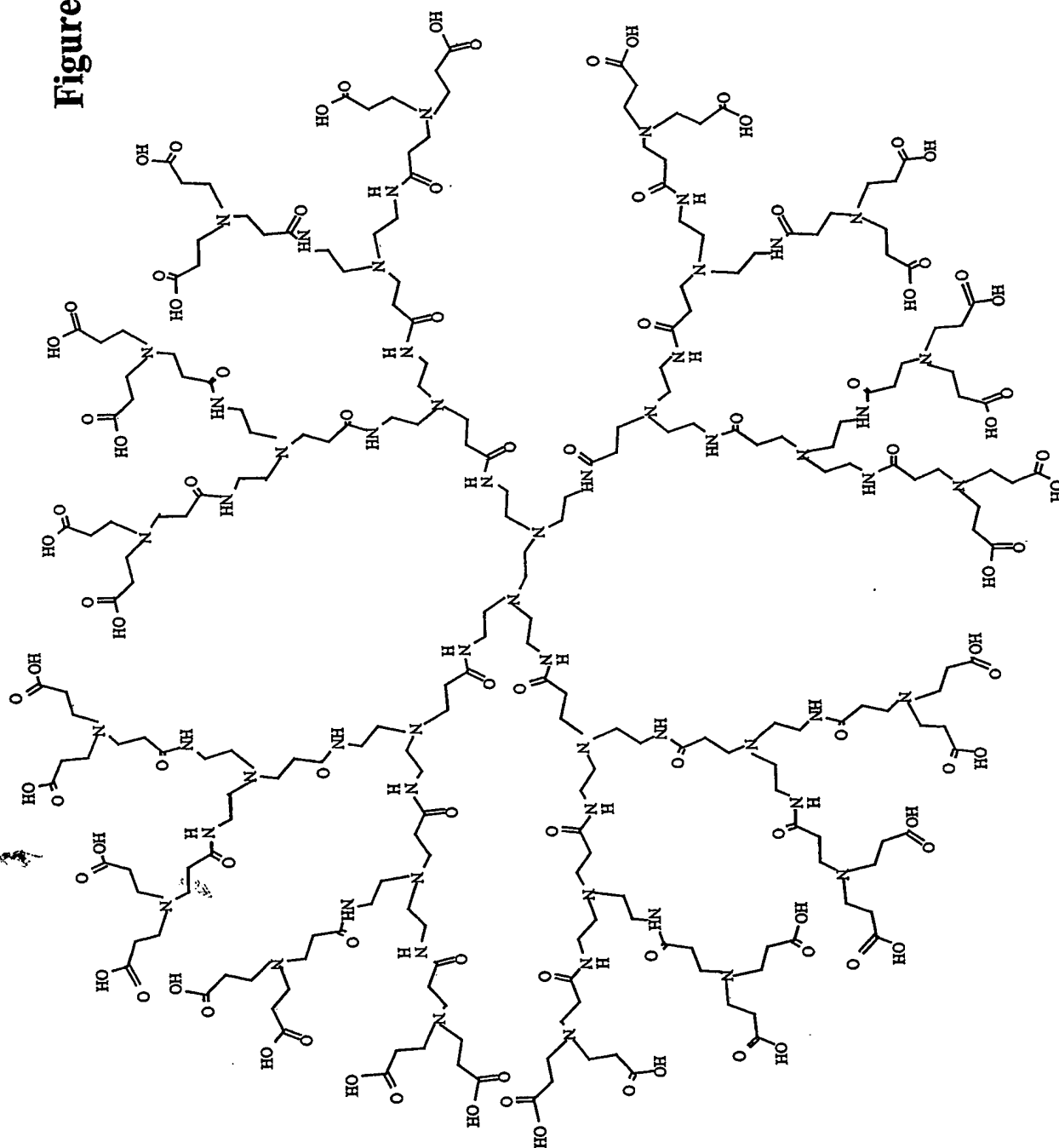
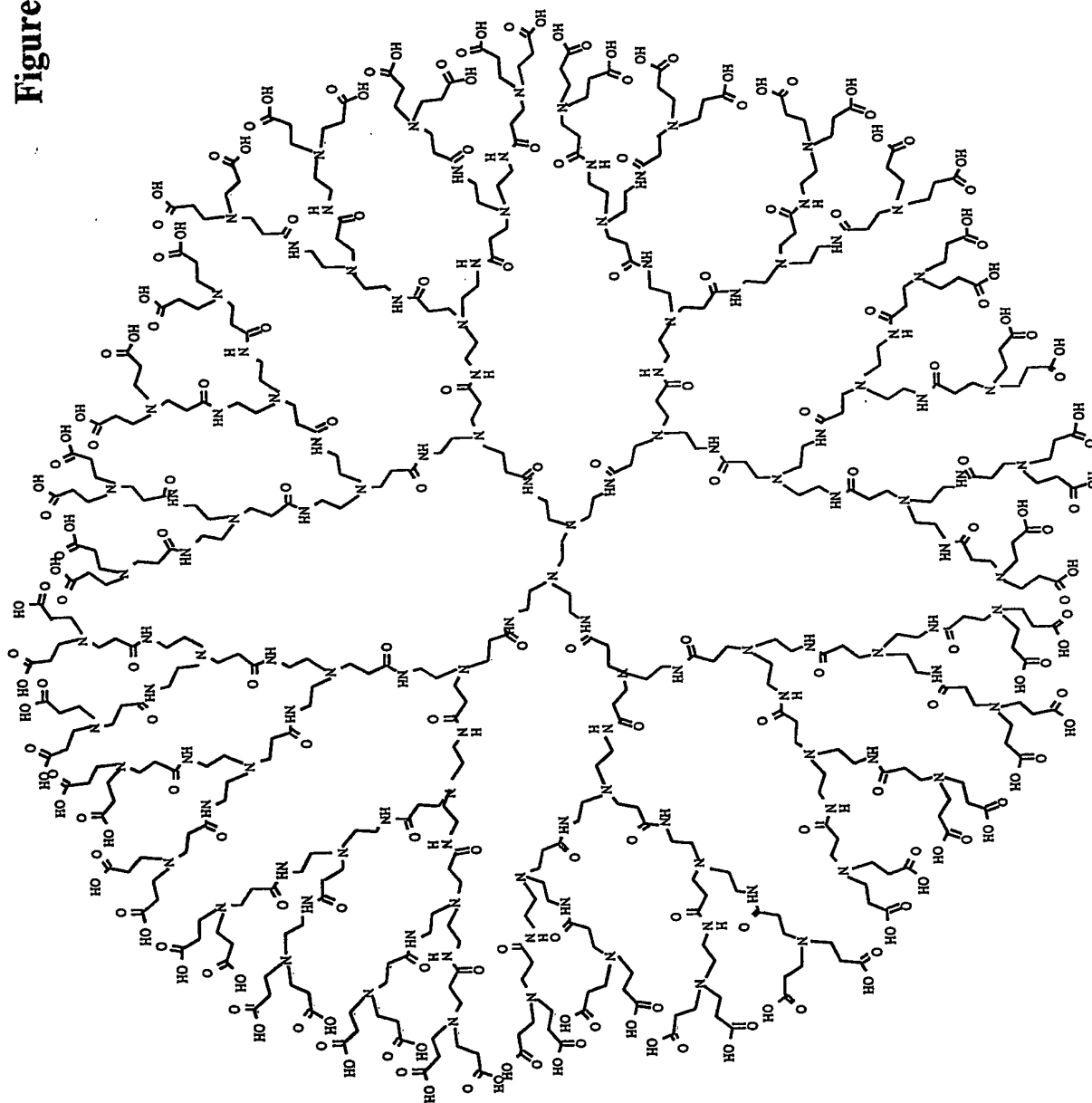


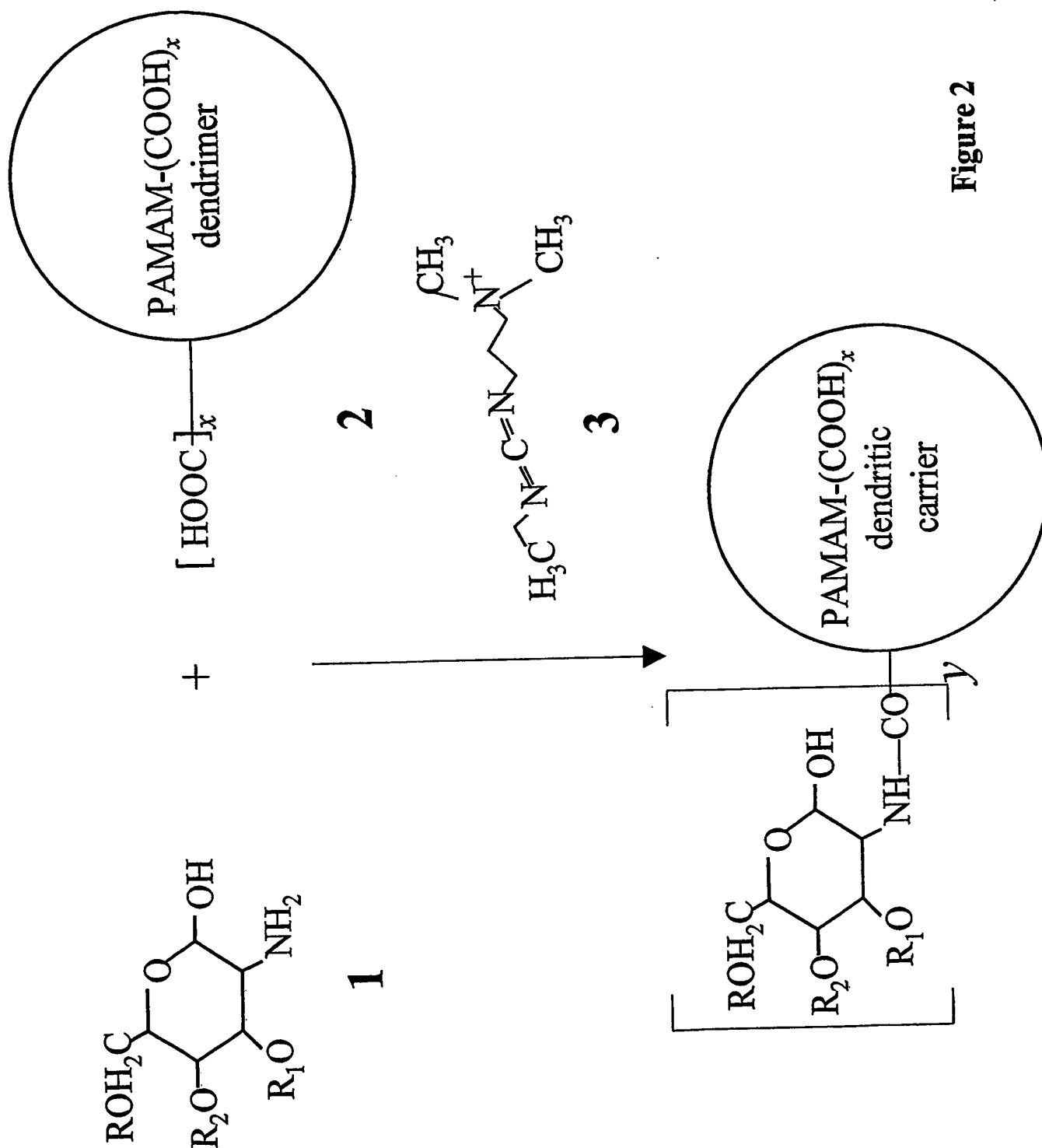
Figure 1a



Best Available Copy

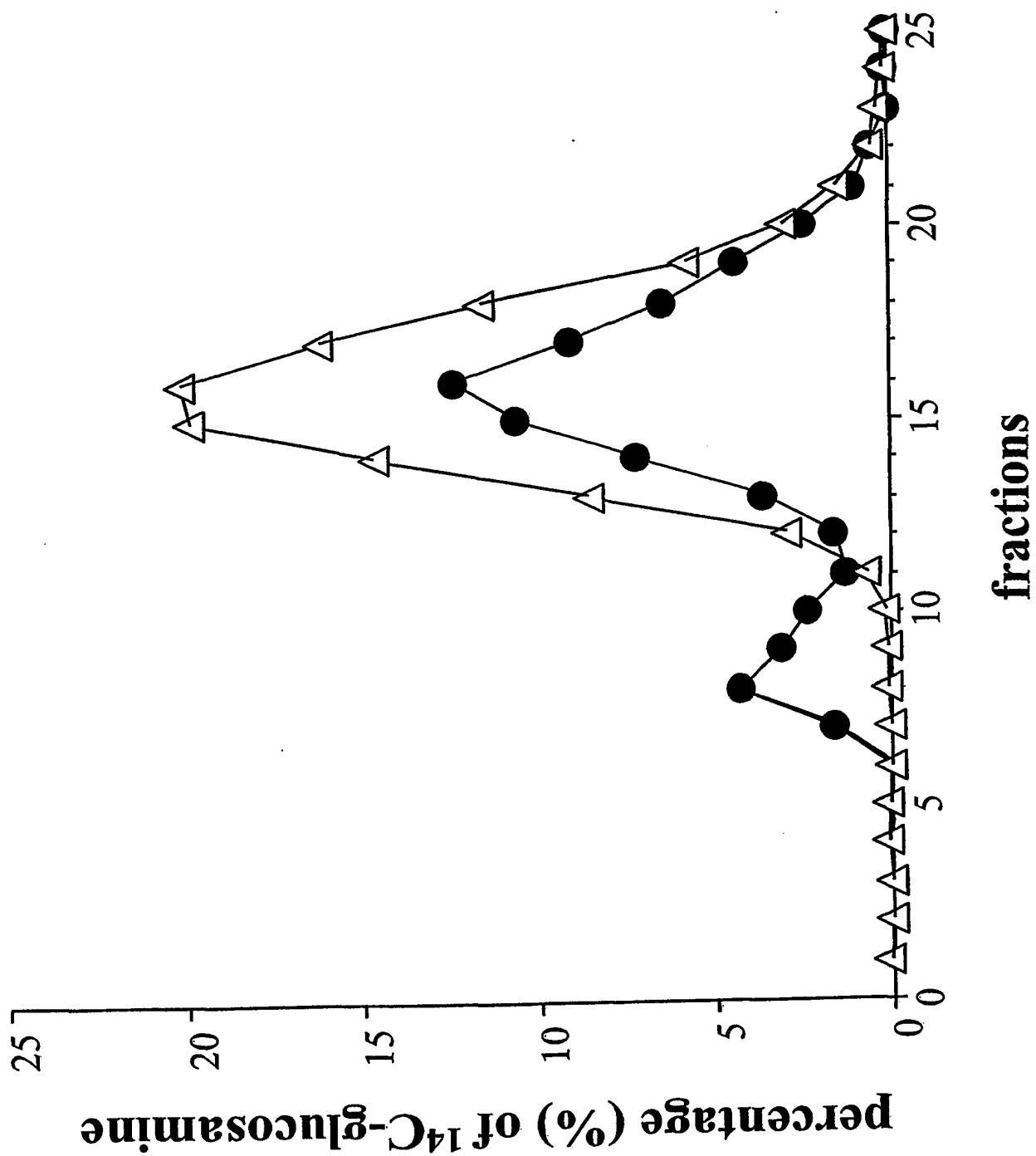
Figure 1b





## Figure 2

Figure 3



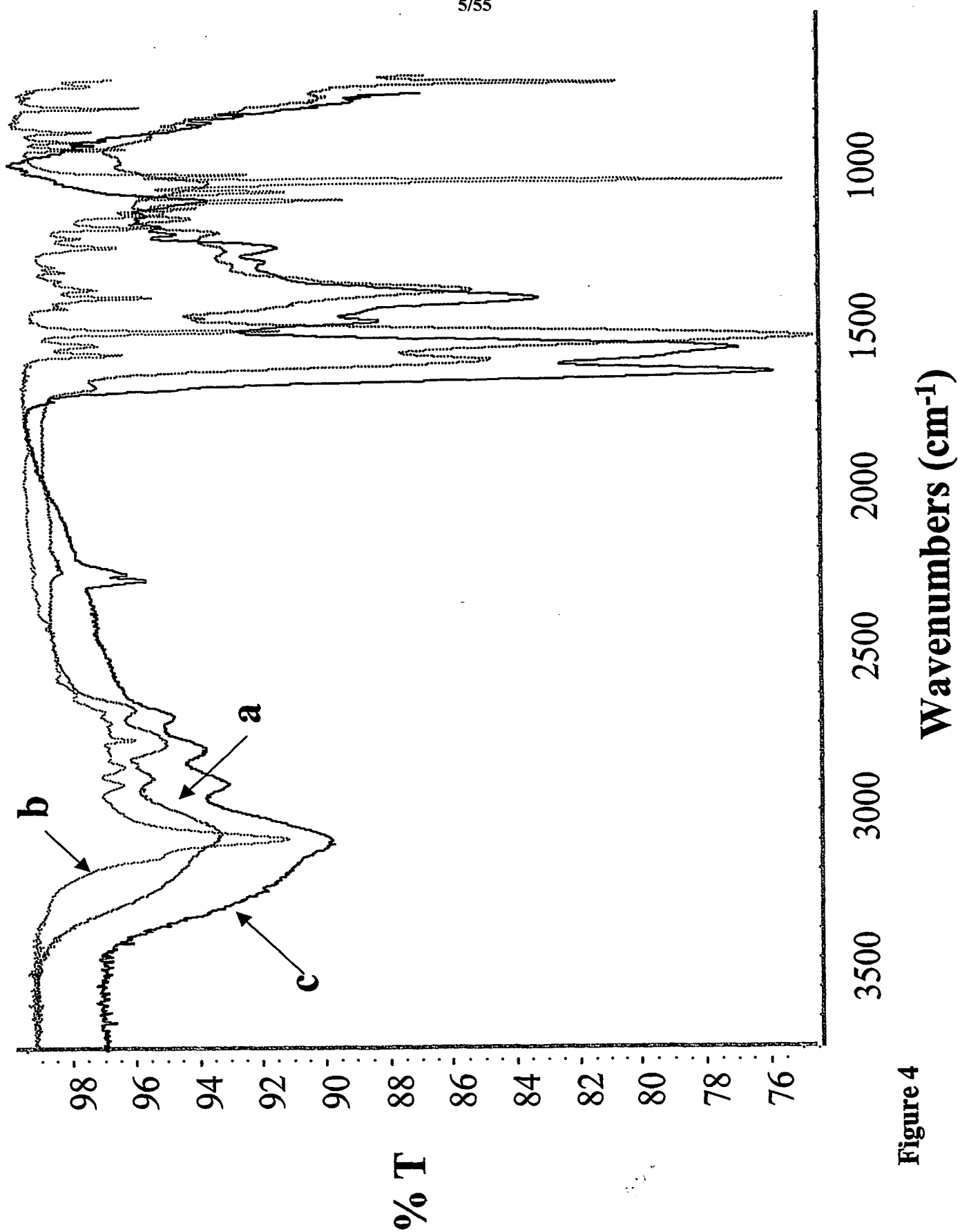
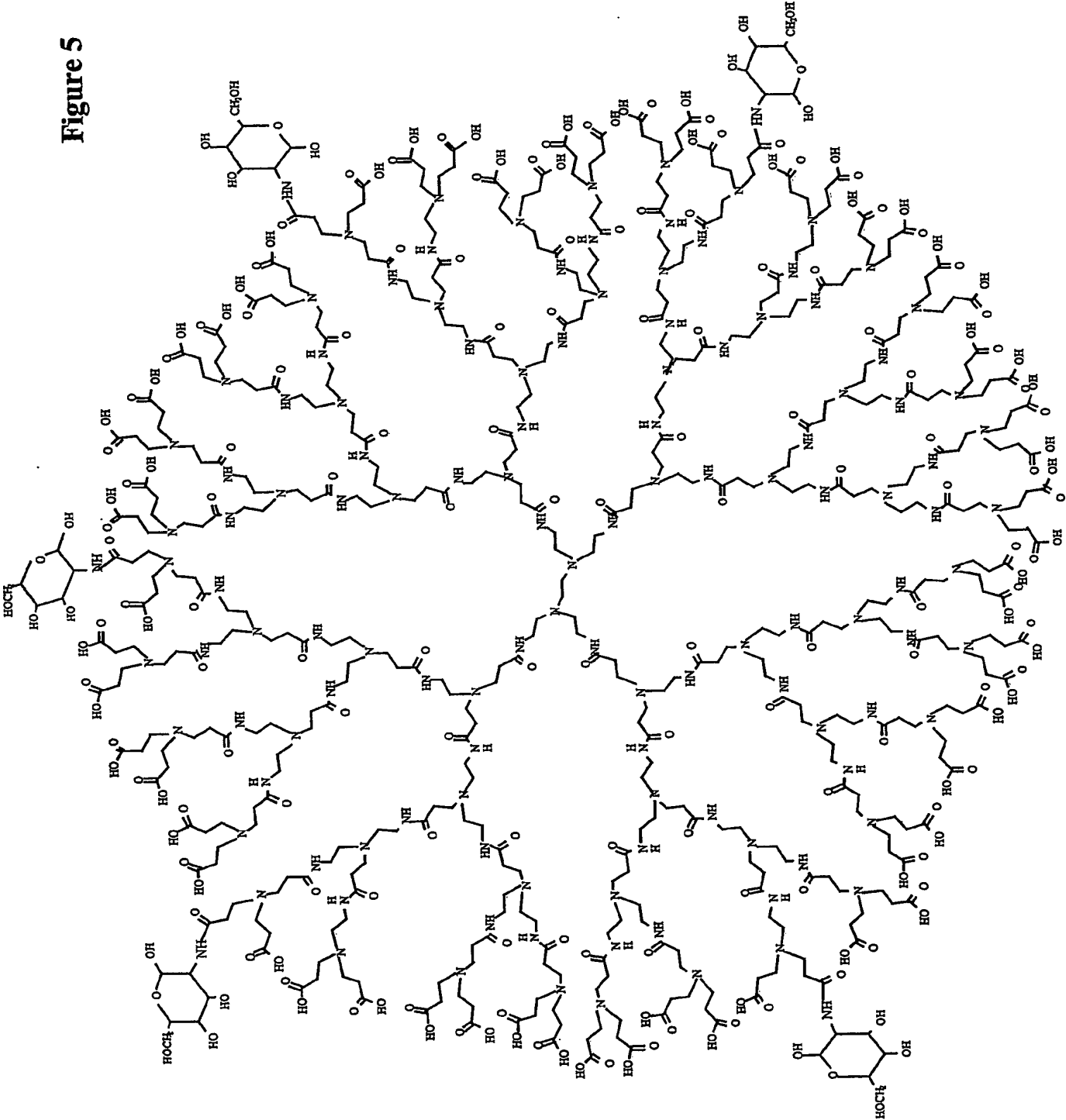


Figure 4

Figure 5



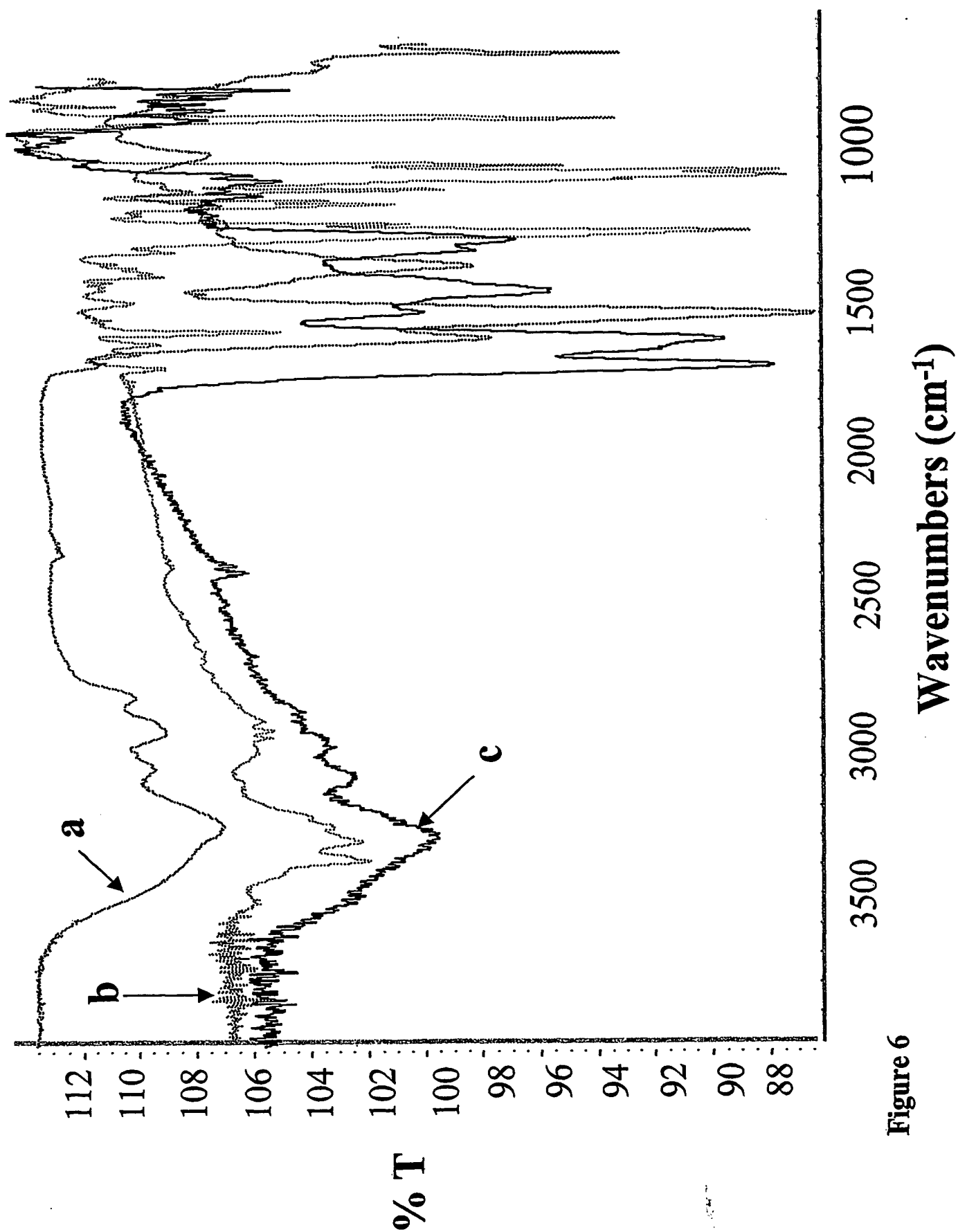


Figure 6

# Percentage (%) of $^{14}\text{C}$ -glucosamine

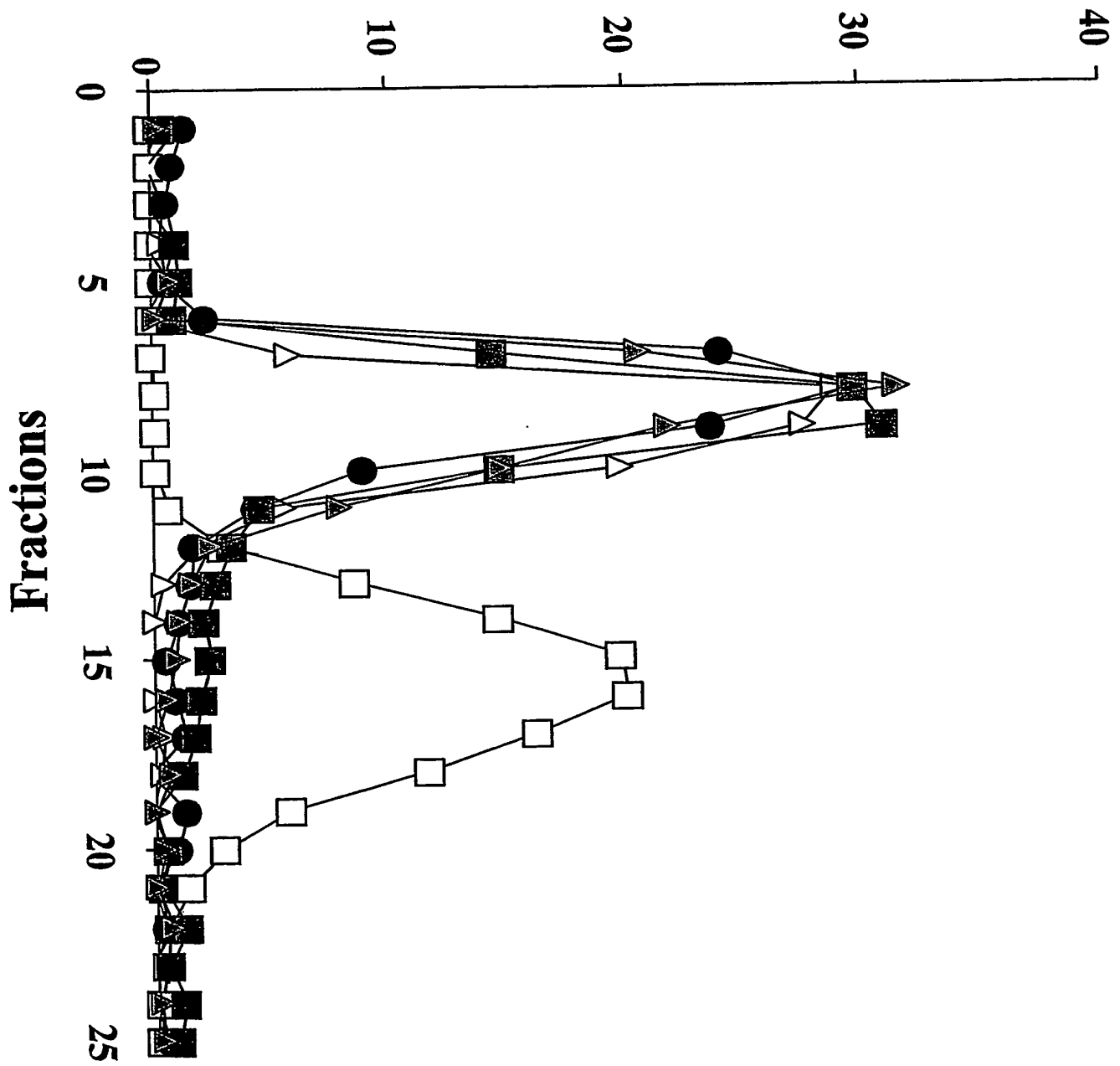


Figure 7(i)



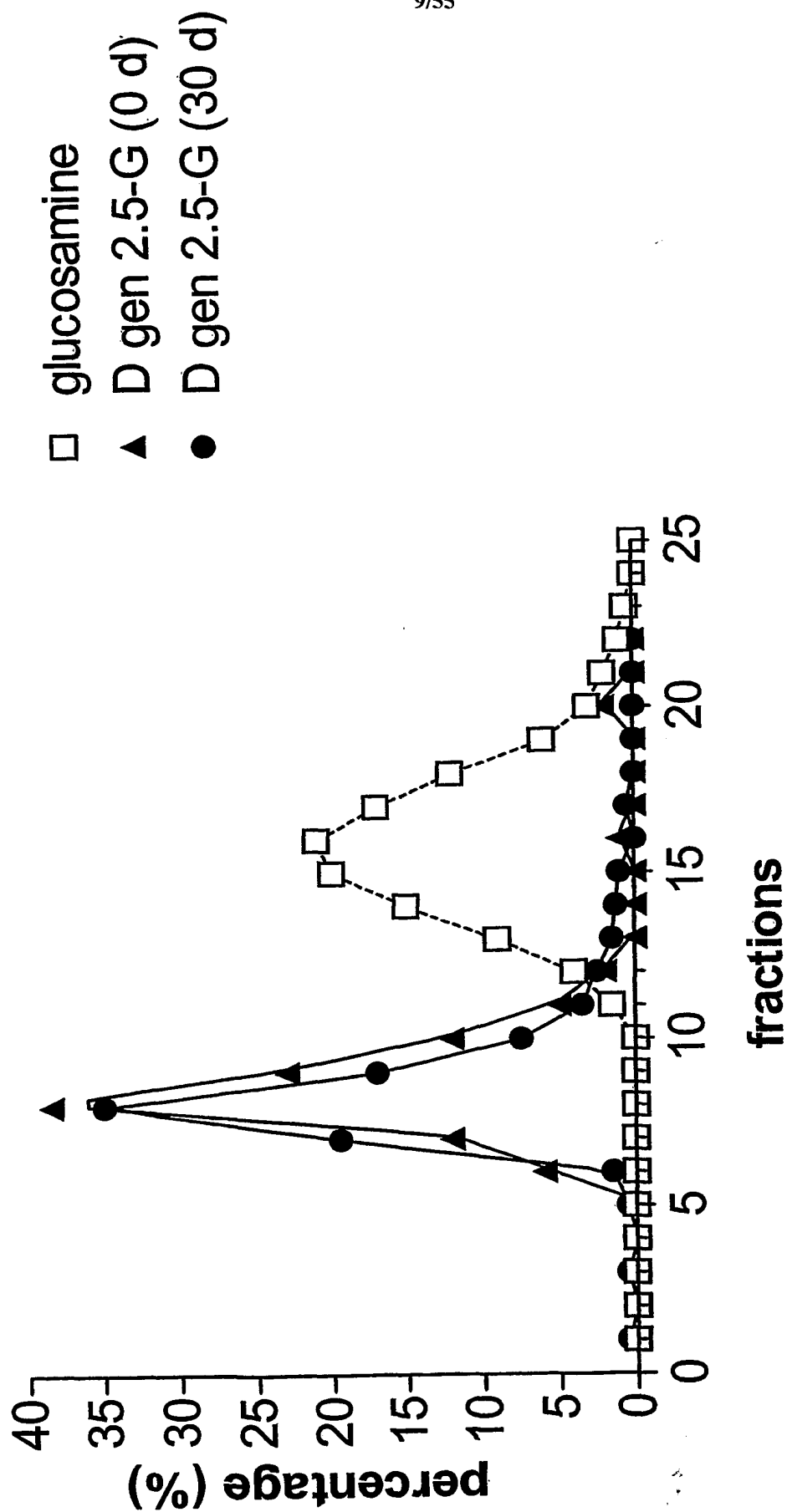
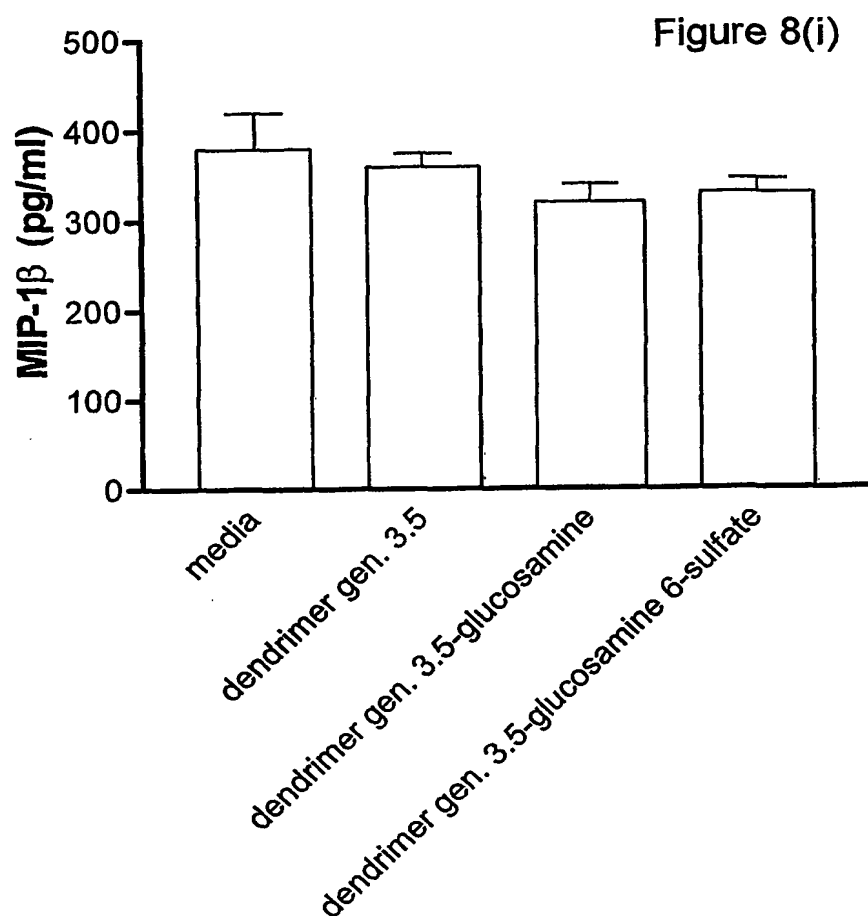
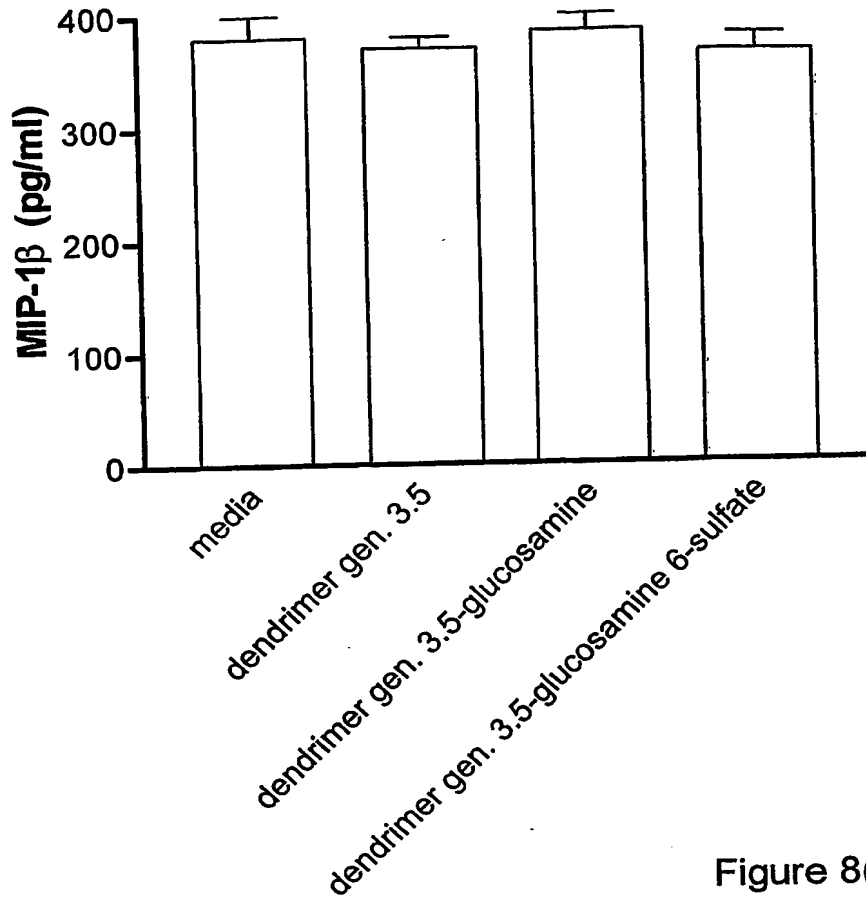


Figure 7(ii):  
PD-10 column of dendrimers gen 2.5-glucosamine after synthesis [D gen 2.5-G (0 d)] and after 30 days of storage [D gen 2.5-G (30 d)]



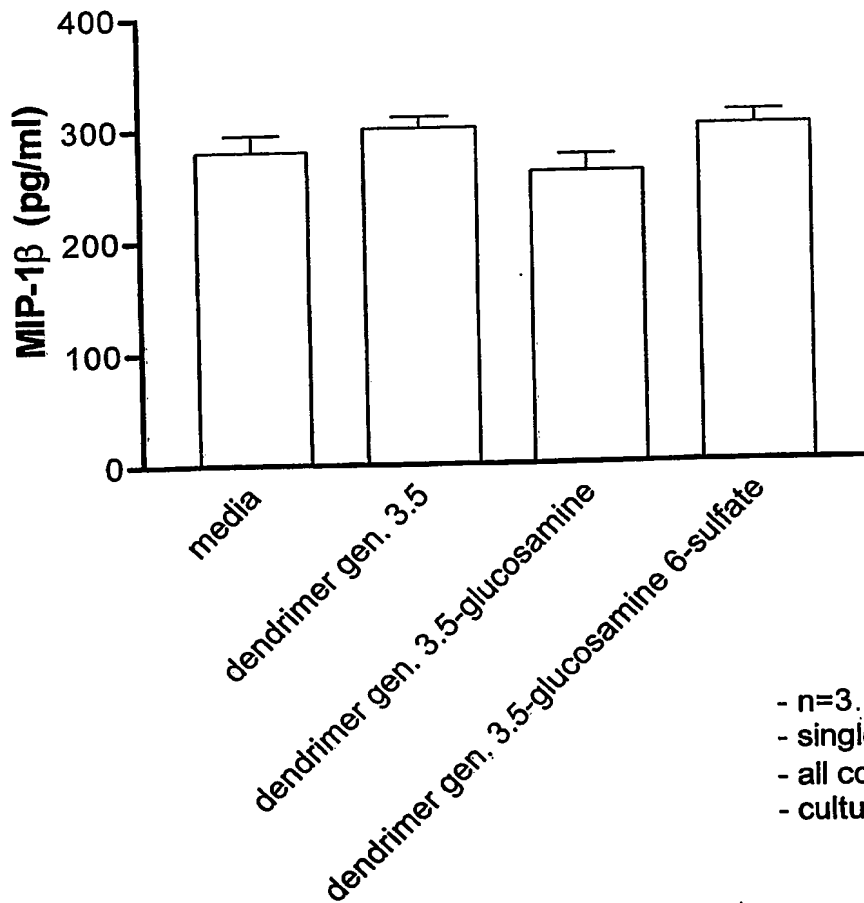
- n = 3. Results shown as mean  $\pm$  sem. p = ns.
- single donor PBMN cells.
- all compounds used at 50  $\mu$ g/ml.
- culture supernatants harvested for MIP-1 $\beta$  at 36 h.

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p = ns

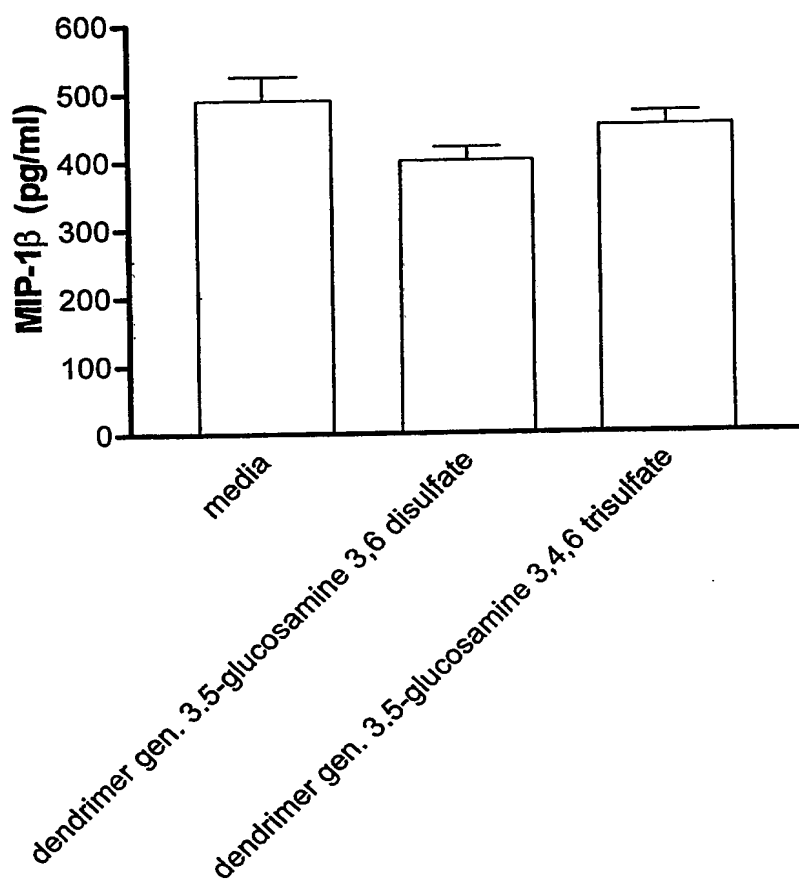
Figure 8(ii)



p = ns

- n=3.
- single donor MDMs.
- all compounds used at 50  $\mu$ g/ml.
- culture s/n harvested at 36 h.

Figure 9



- n = 3.
- single donor MDMs.
- all compounds used at 50  $\mu$ g/ml.
- culture supernatants harvested for MIP-1 $\beta$  after 36 h.

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Figure 10(i)

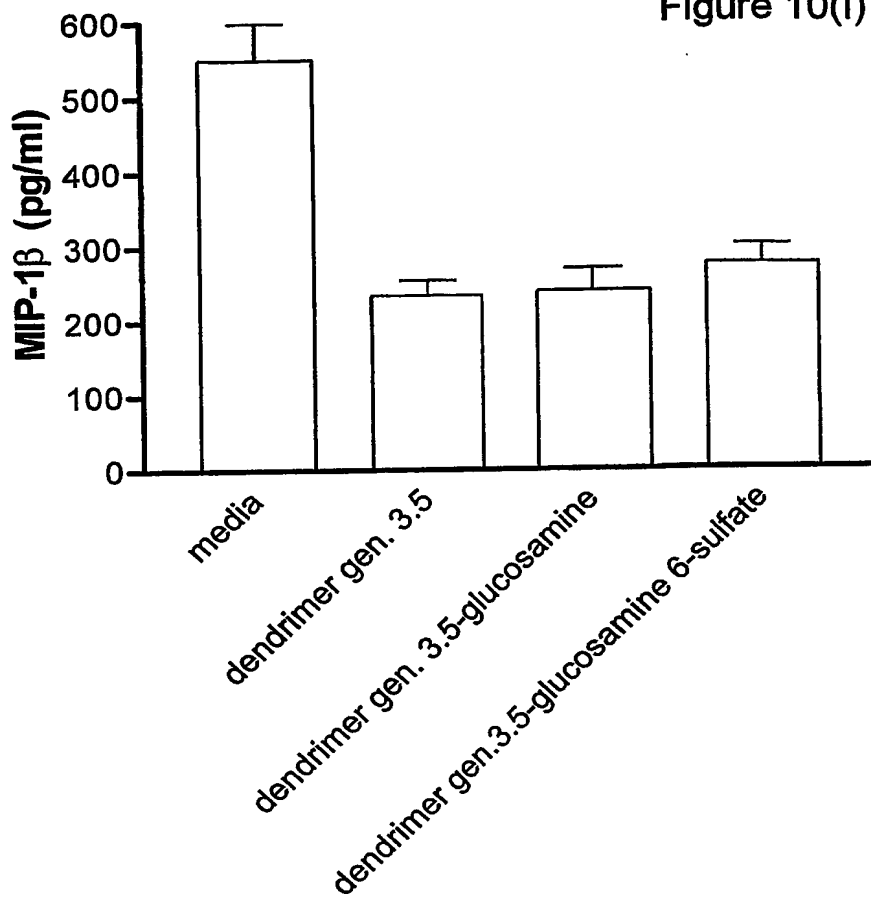
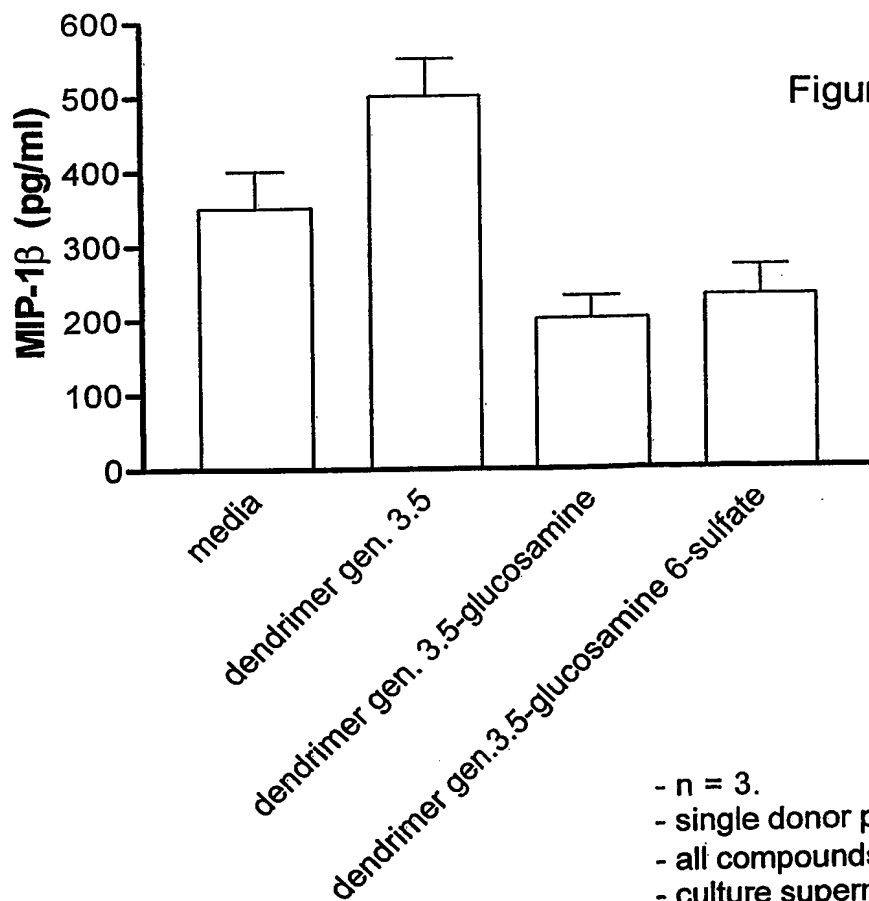


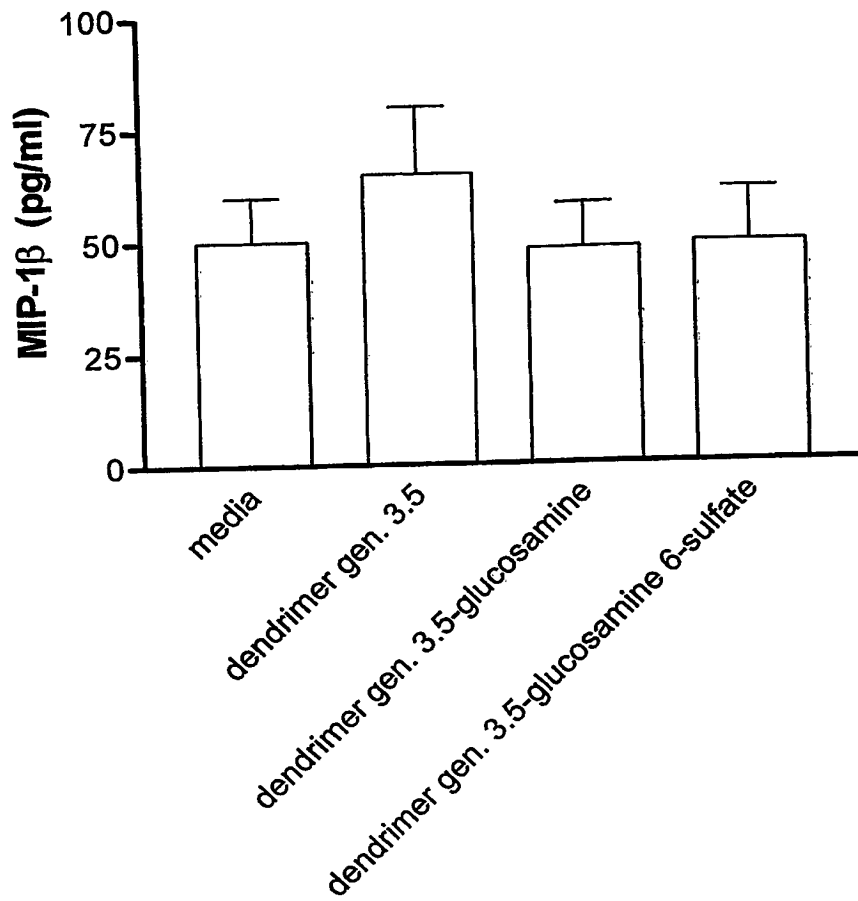
Figure 10(ii)



p = ns

- n = 3.
- single donor peritoneal macrophages.
- all compounds used at 25  $\mu$ g/ml.
- culture supernatants harvested at 36 h.

Figure 10(iii)



- n = 3.
- single donor peritoneal macrophages.
- all compounds used at 25  $\mu$ g/ml.
- culture supernatants harvested for MIP-1 $\beta$  at 72 h.

Figure 11(i)

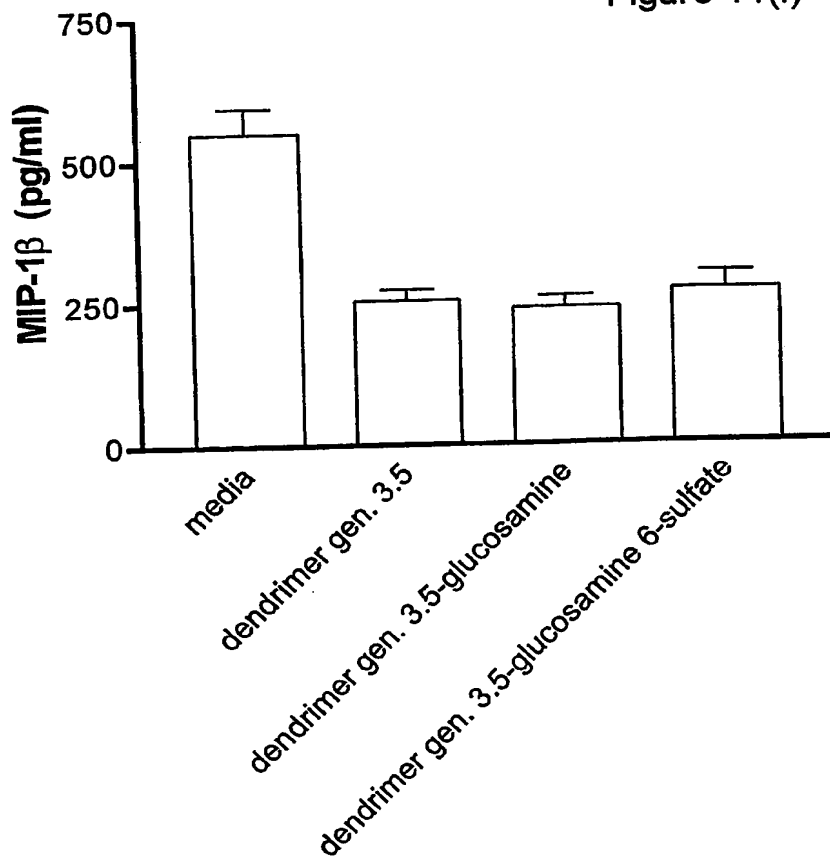
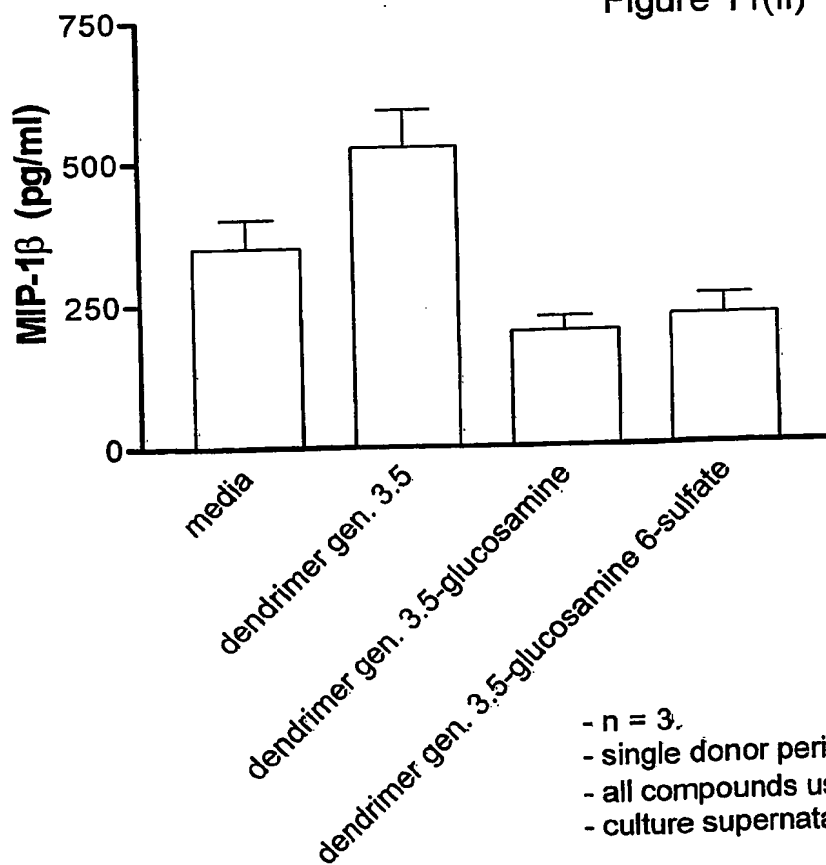


Figure 11(ii)



p = ns

- n = 3.
- single donor peritoneal macrophages.
- all compounds used at 50 µg/ml.
- culture supernatants harvested at 36 h.

Figure 12 (i)

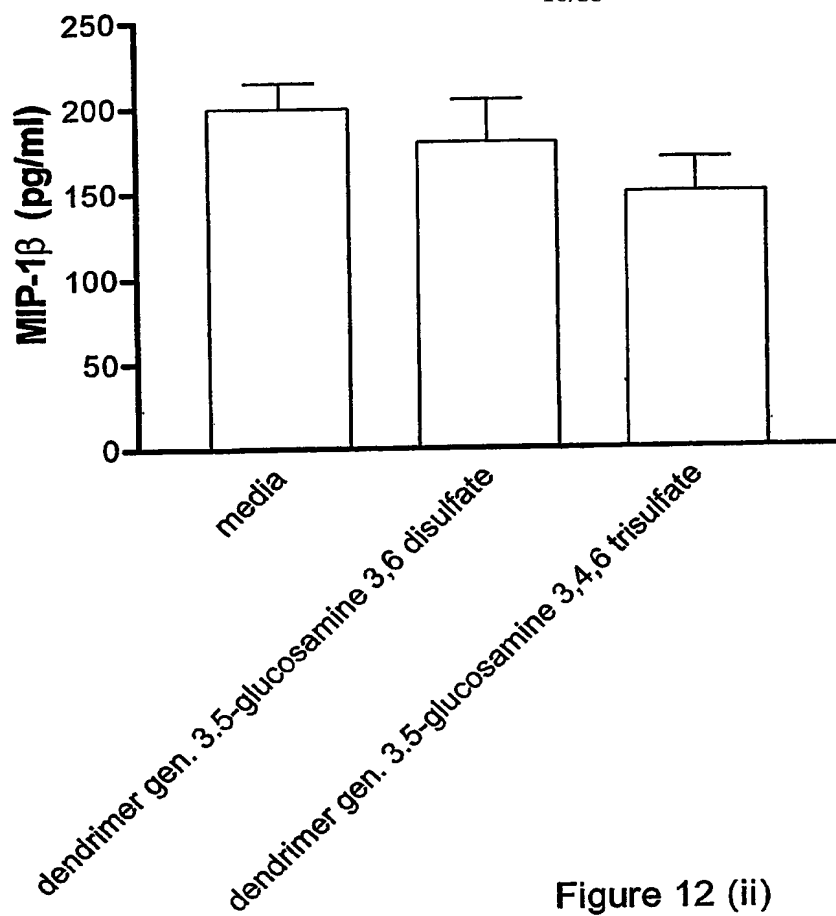
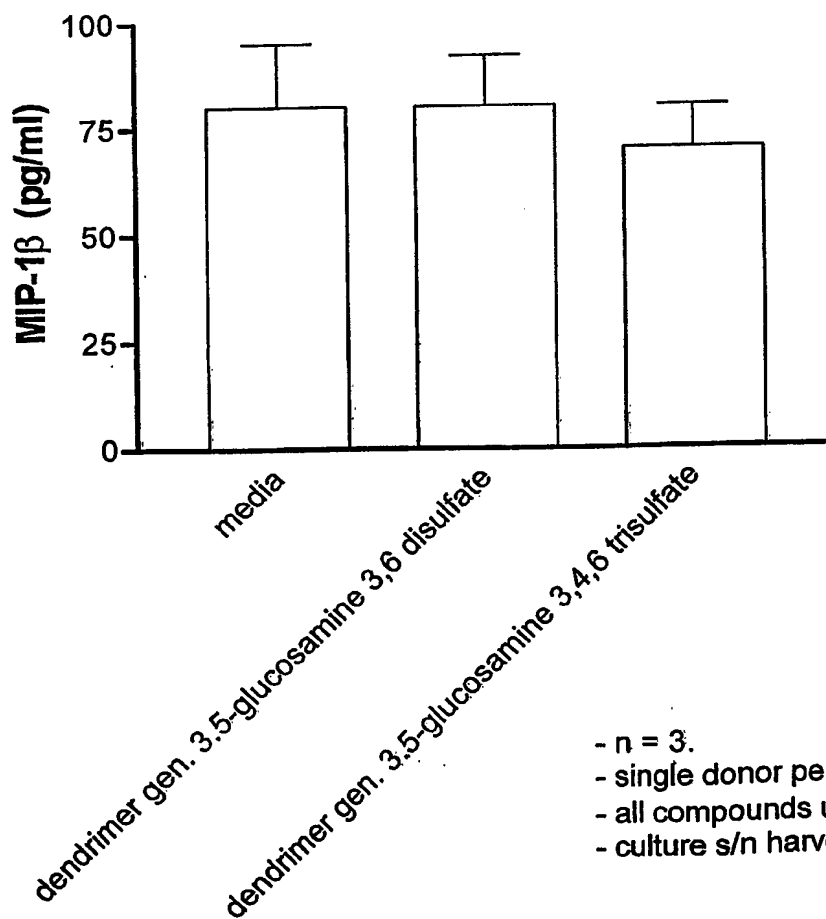
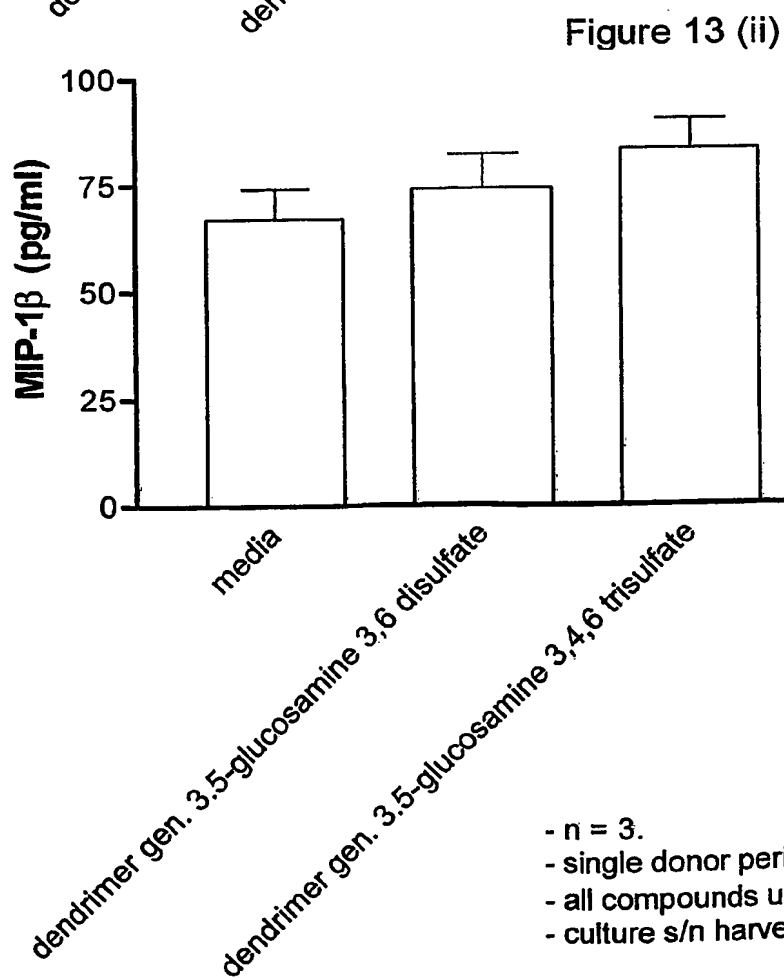
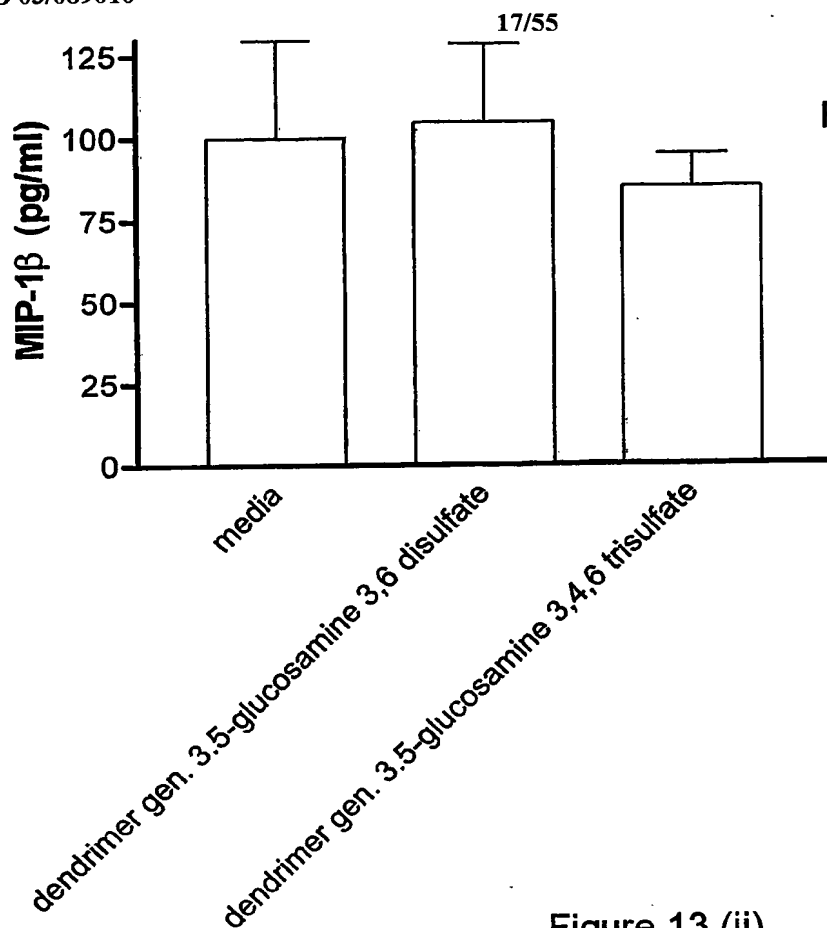


Figure 12 (ii)



- n = 3.
- single donor peritoneal macrophages.
- all compounds used at 50  $\mu$ g/ml.
- culture s/n harvested at 36 h.





- n = 3.
- single donor peritoneal macrophages.
- all compounds used at 100  $\mu$ g/ml.
- culture s/n harvested at 36 h.

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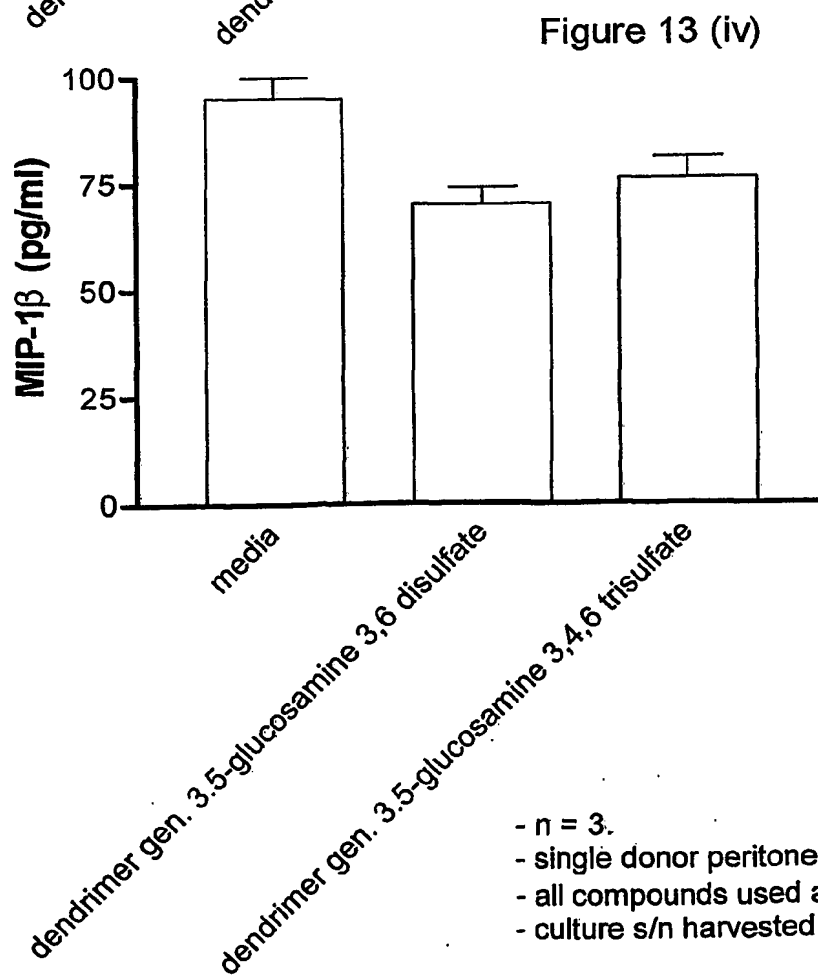
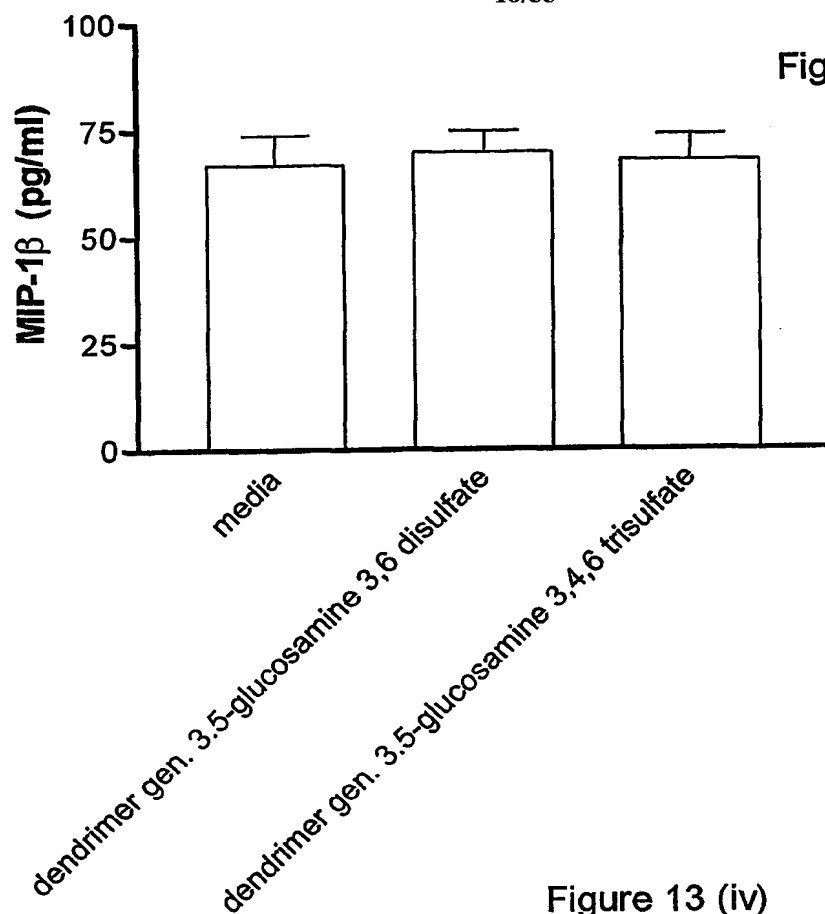
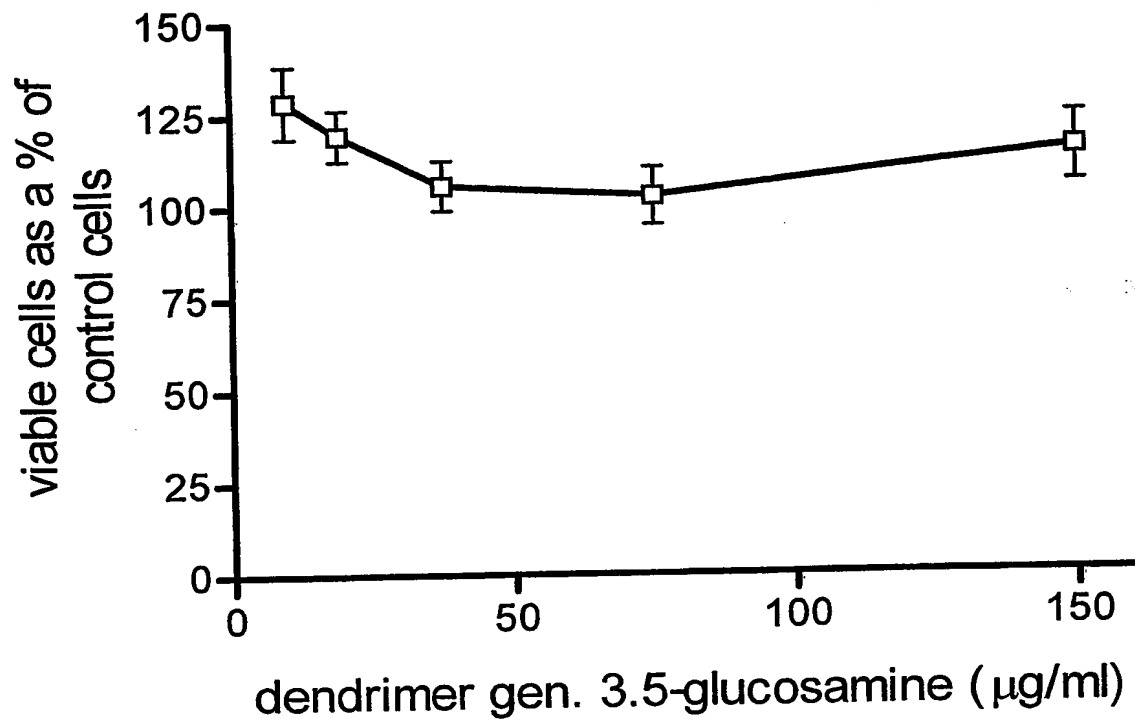
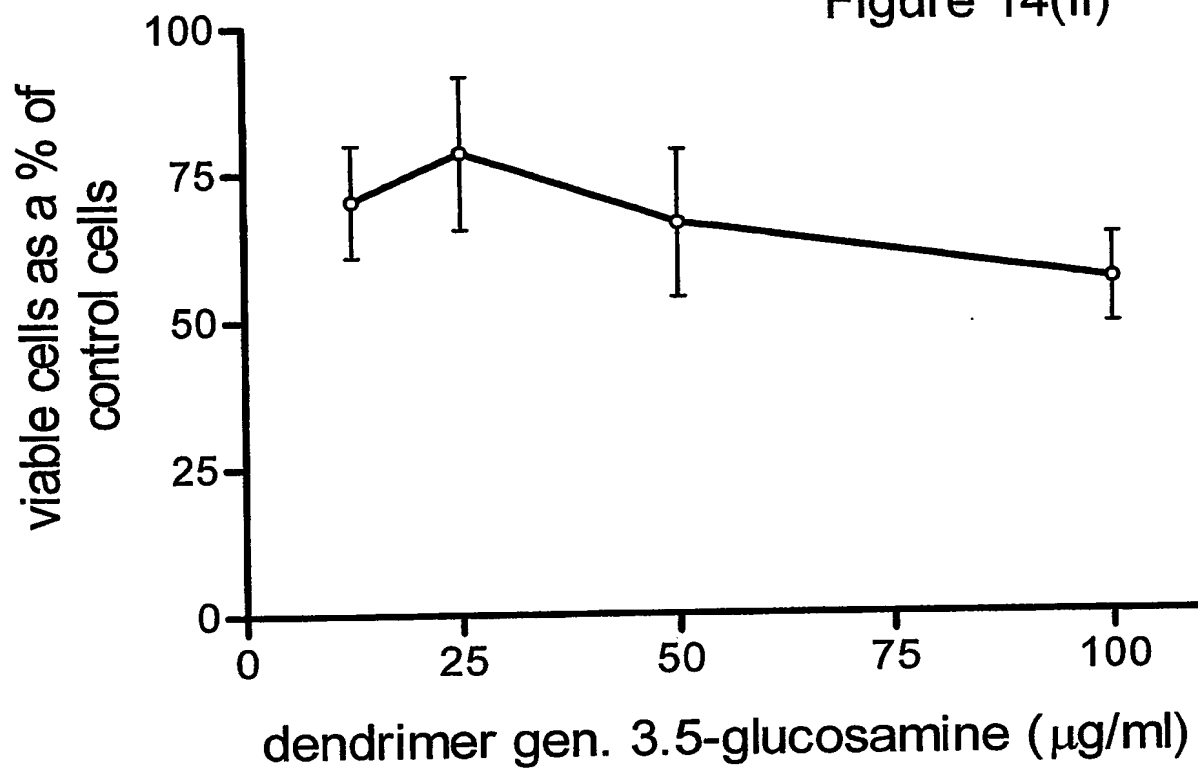


Figure 14(i)



- n = 3.
- single donor MDMs.
- MTT assay.

Figure 14(ii)



- n = 3.
- HUVECs.
- MTT assay.

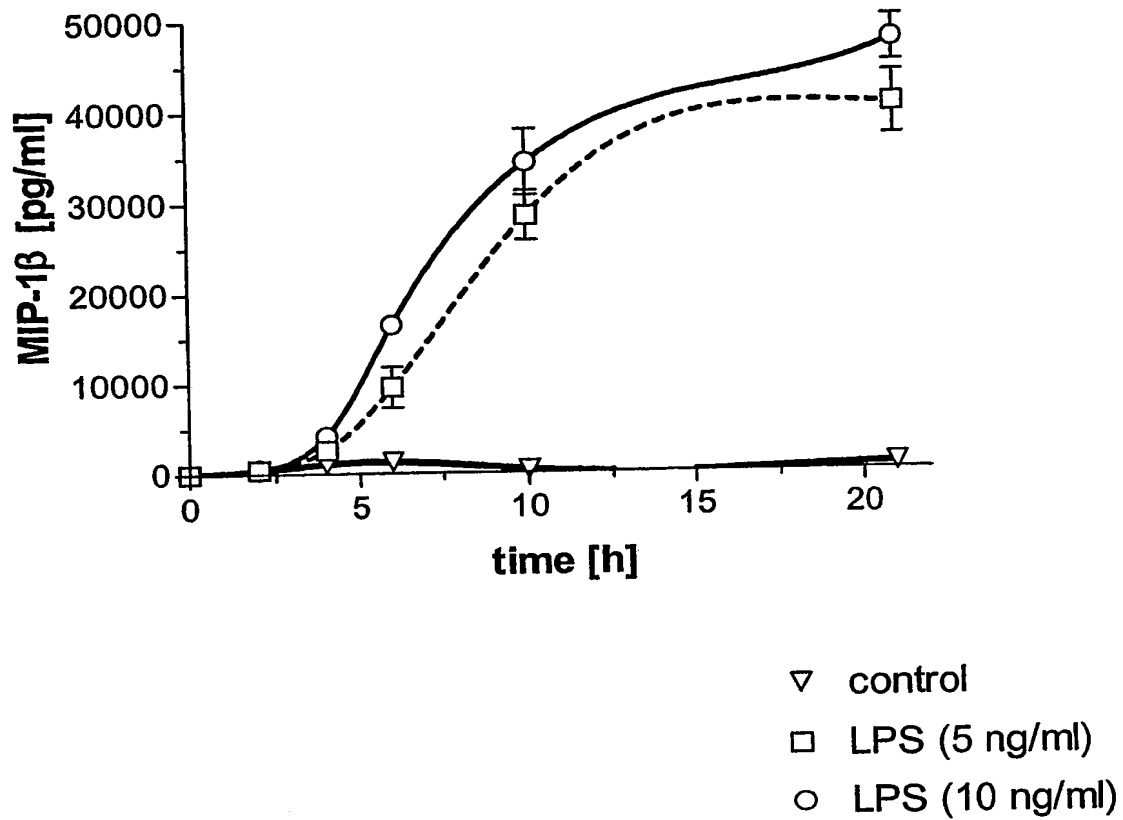
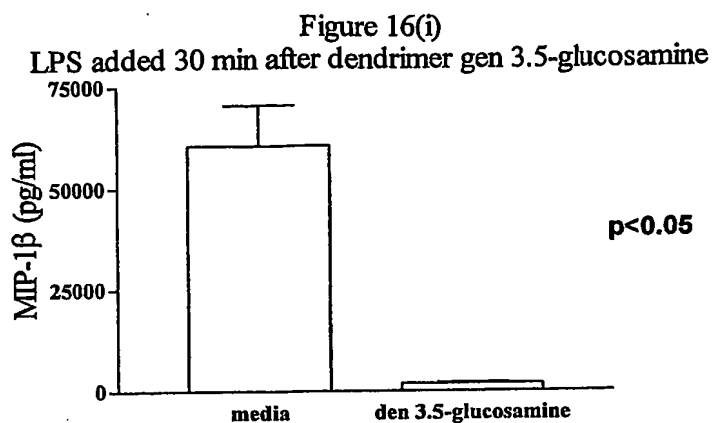
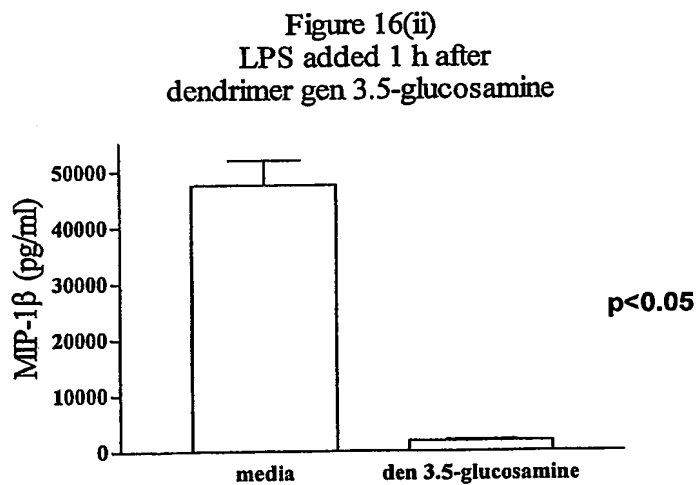


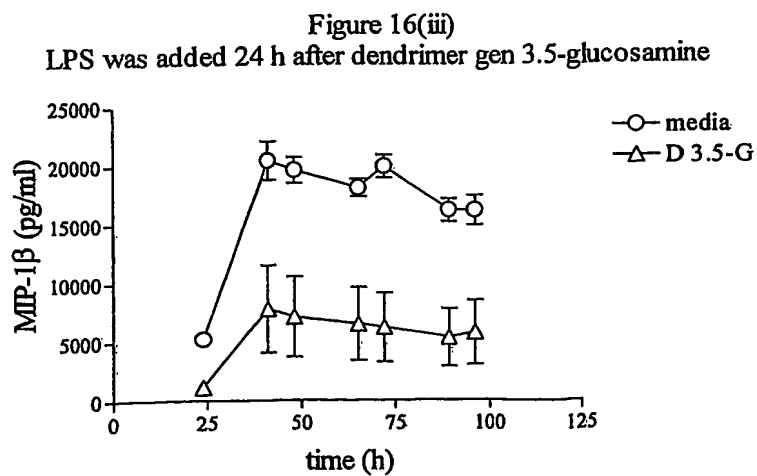
Figure 15:  
Time course of the release of MIP-1 $\beta$  into cell free culture supernatants  
after the addition of LPS at a final concentration of 5 ng/ml & 10 ng/ml.



- n = 4
- mean  $\pm$  sem
- D 3.5-G at 100  $\mu$ g/ml
- LPS at 5 ng/ml
- harvest at 21 h



- n = 4
- mean  $\pm$  sem
- D 3.5-G at 100  $\mu$ g/ml
- LPS at 5 ng/ml
- harvest at 21 h



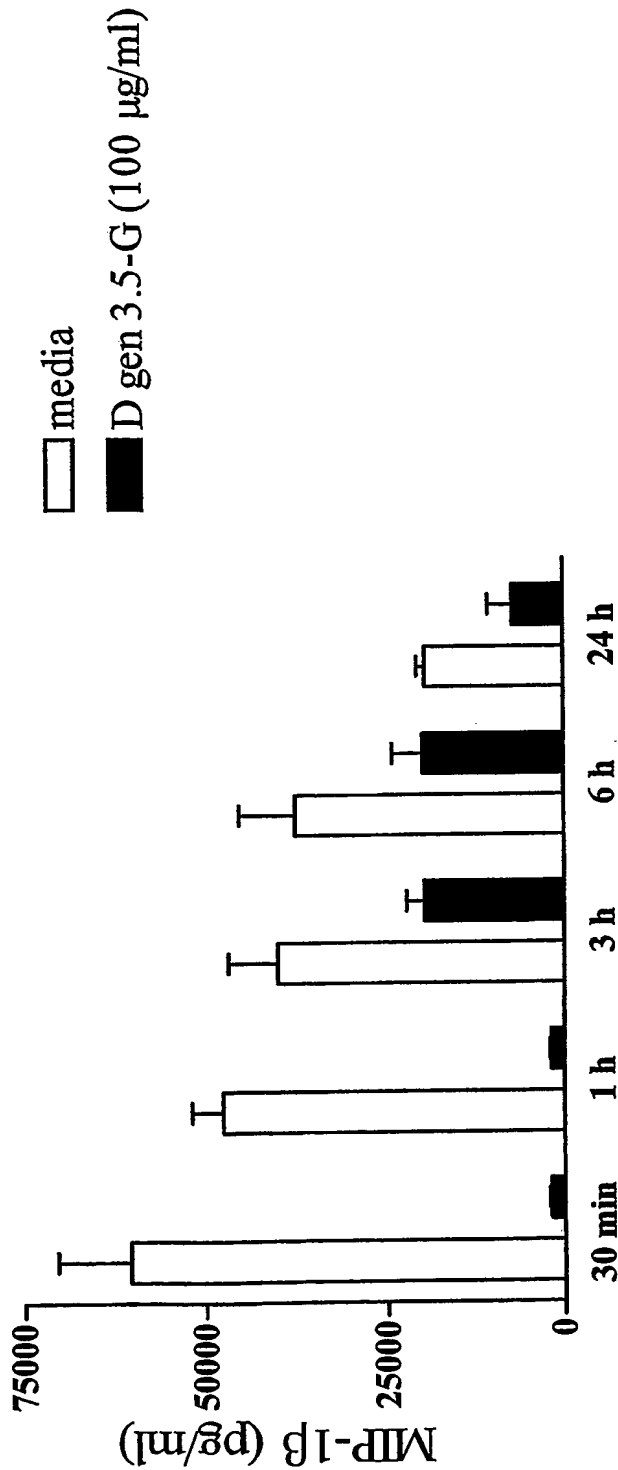


Figure 16(iv)

- Release of MIP-1β from single donor PBMN cells. n = 4.
- LPS (5 ng/ml) was added at various time points ranging from 30 min to 24 h after dendrimer gen. 3.5-glucosamine (100 µg/ml).
- Culture supernatants harvested 21 h after the addition of LPS for MIP-1 β.

(D gen 3.5-G: dendrimer gen 3.5-glucosamine)

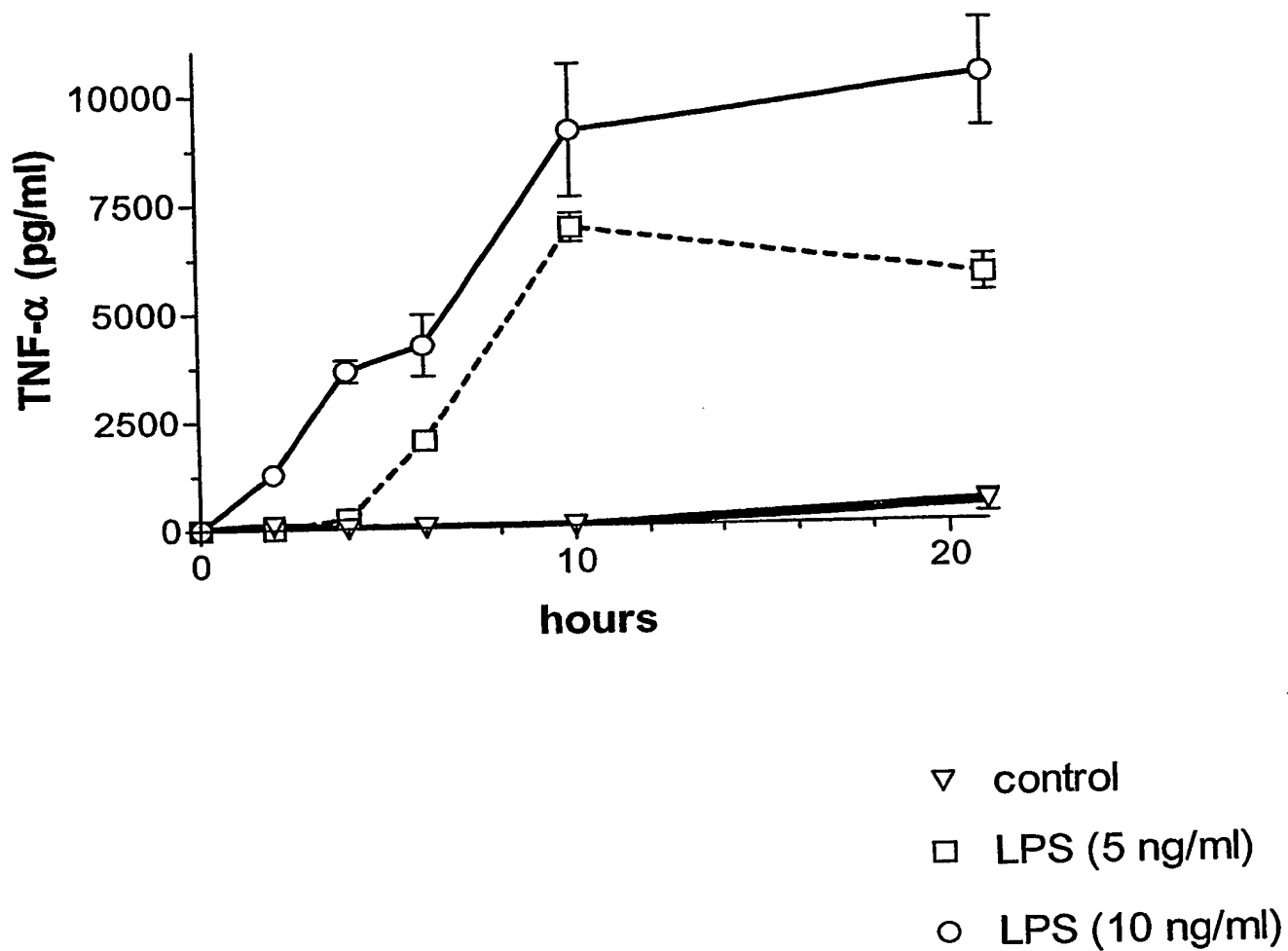


Figure 17:  
Time course of the release of TNF- $\alpha$  into cell free culture supernatants following the addition of LPS at a final concentration of 5 ng/ml and at 10 ng/ml.



Figure 18:  
LPS (5 ng/ml) was added 30 min after dendrimer gen 3.5-glucosamine (100 µg/ml) and culture supernatants harvested at 21 h.  
(P<0.05 for all 6 figures)

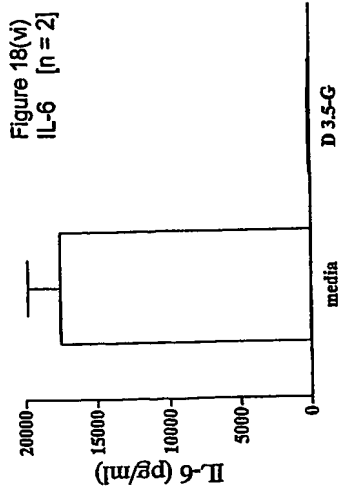
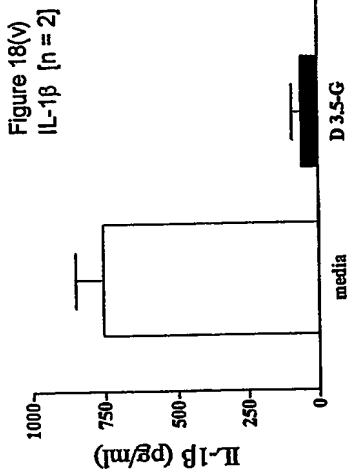
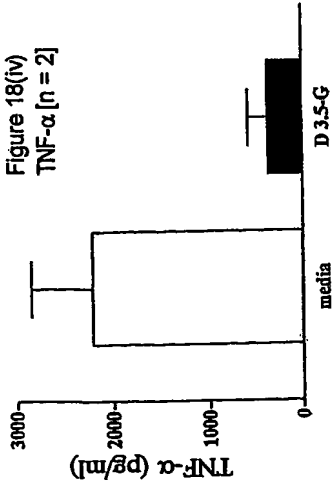
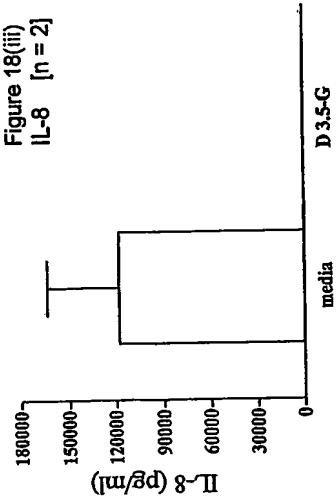
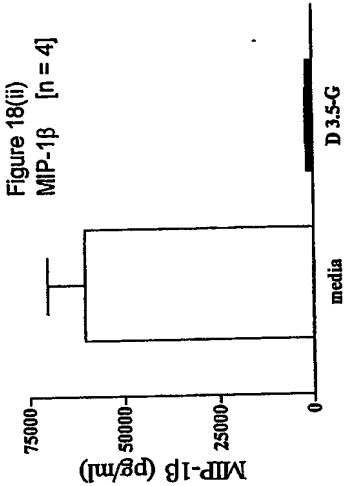
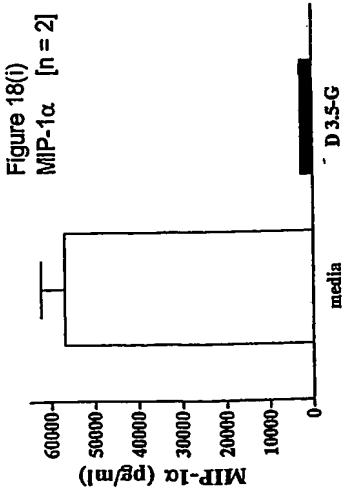


Figure 19:

LPS (5 ng/ml) was added 30 min after dendrimer gen 3.5-glucosamine (200 µg/ml) and culture supernatants harvested at 25 h. (P<0.05 for all groups)

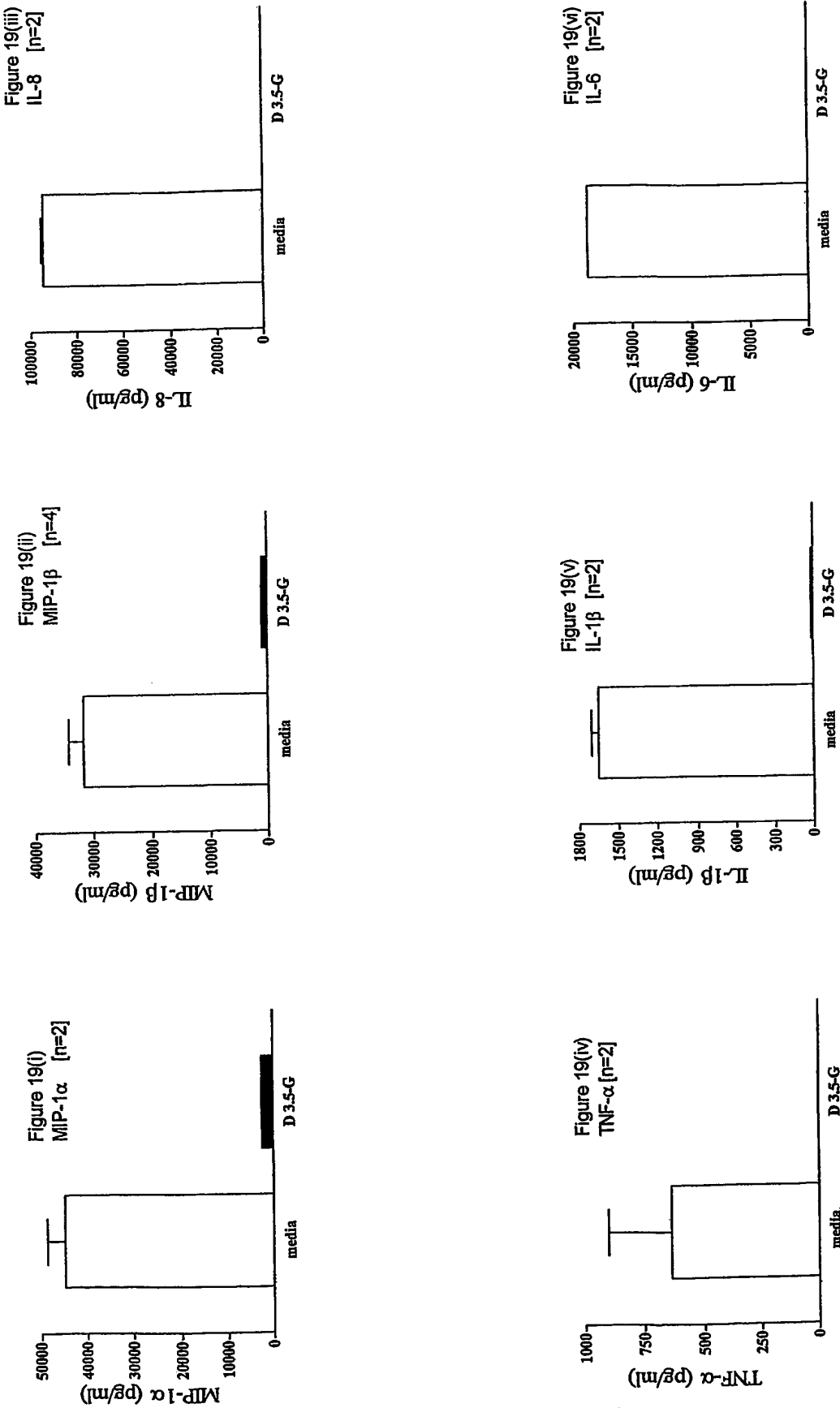
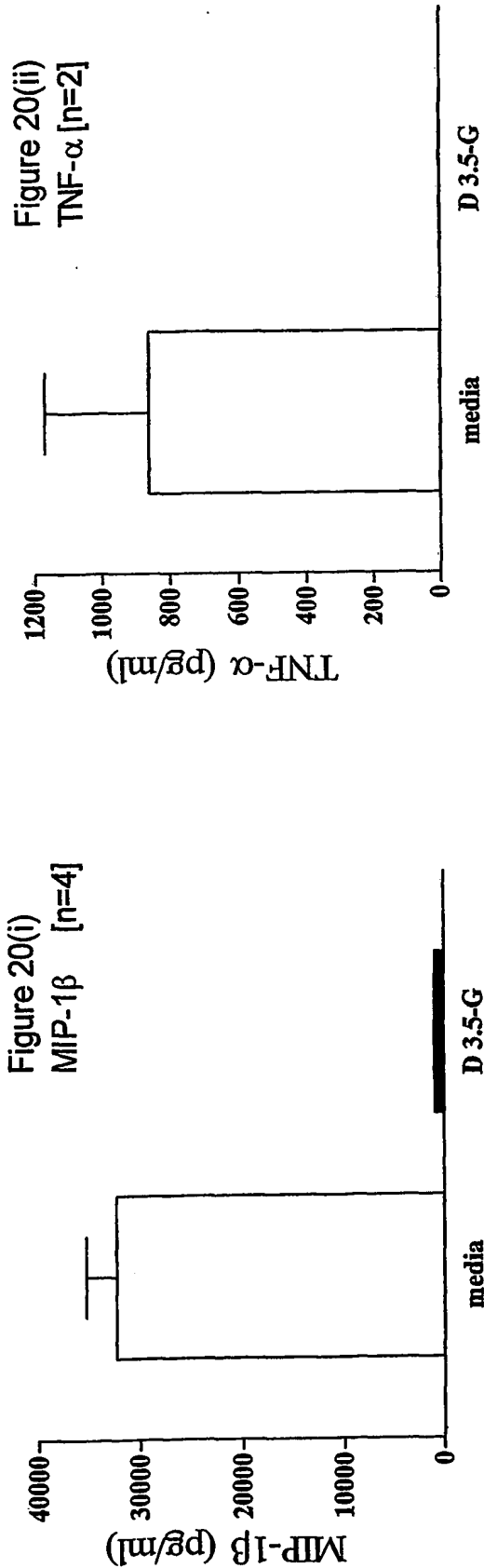


Figure 20:  
LPS (5 ng/ml) added 1 hour after dendrimer gen 3.5-glucosamine (200 µg/ml) and culture supernatants harvested at 25 h.  
(P<0.05 for all groups)



mean ± sem

Figure 21(i): Dendrimer gen 3.5-glucosamine ((D 3.5 G at 100  $\mu$ g/ml) added 30 min or 1 h or 2 h or 3 h or 4 h after LPS (5 ng/ml). Culture supernatants harvested at 21 h.

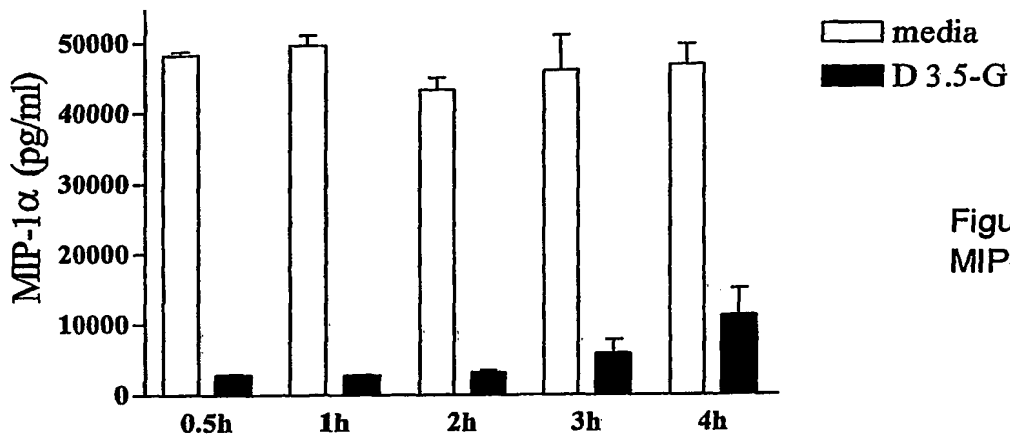


Figure 21(i)  
MIP-1 $\alpha$  [n=2]

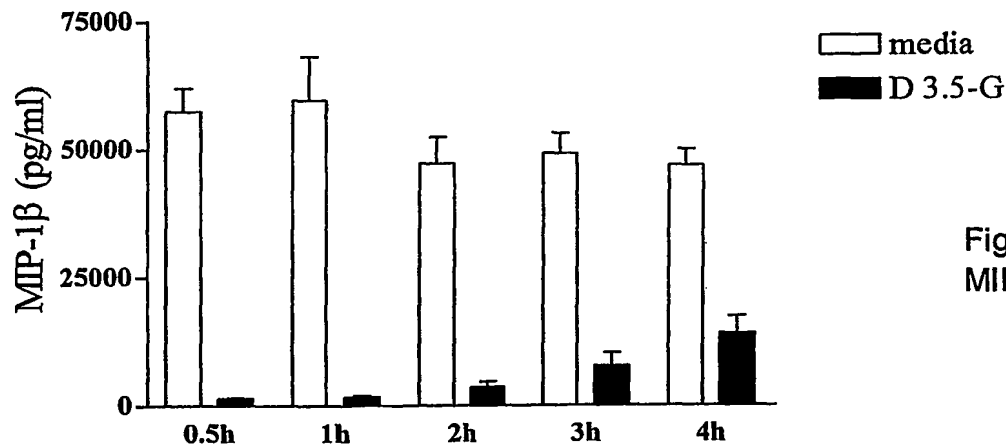


Figure 21(ii)  
MIP-1 $\beta$  [n=4]

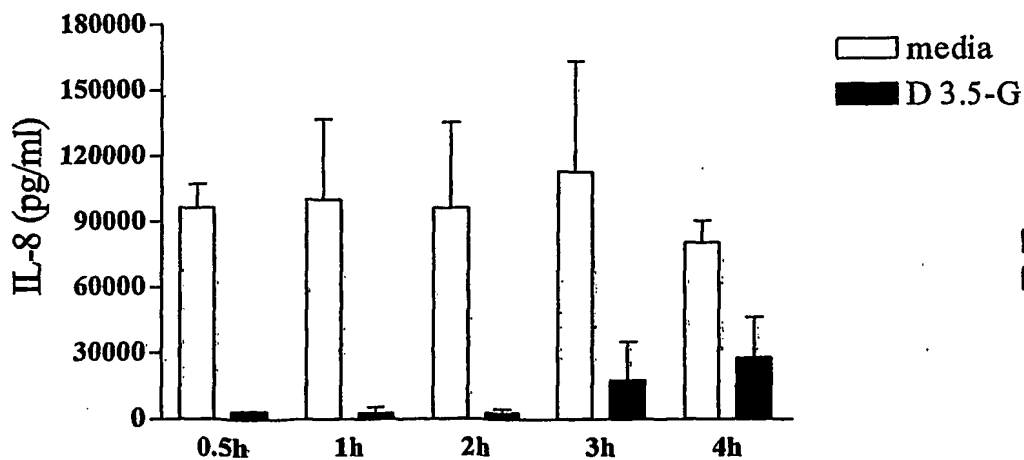


Figure 21(iii)  
IL-8 [n=2]

Figure 21(ii): Dendrimer gen 3.5-glucosamine (100  $\mu\text{g/ml}$ ) added 30 min or 1 h or 2 h or 3 h or 4 h after LPS (5 ng/ml). Culture supernatants harvested at 21 h.

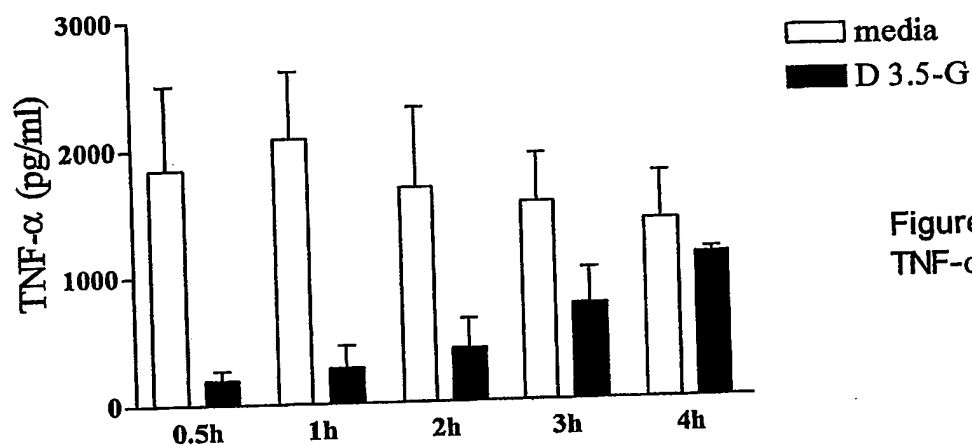


Figure 21(iv)  
TNF- $\alpha$  [n=2]

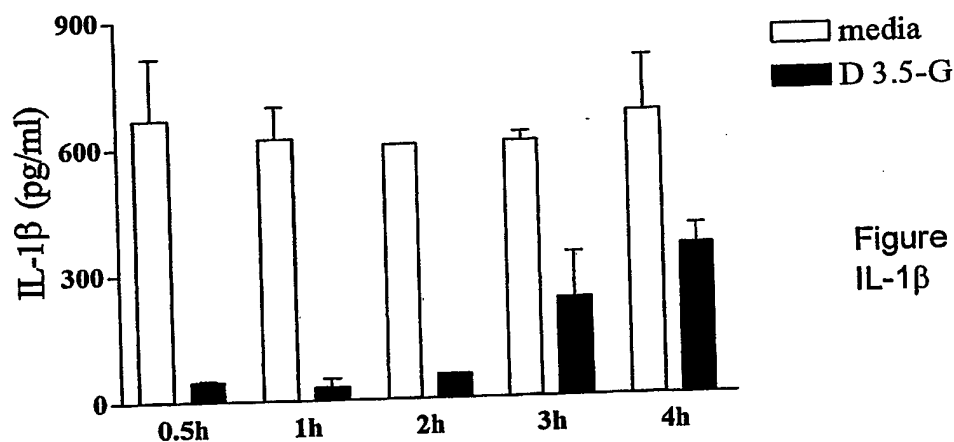


Figure 21(v)  
IL-1 $\beta$  [n=2]

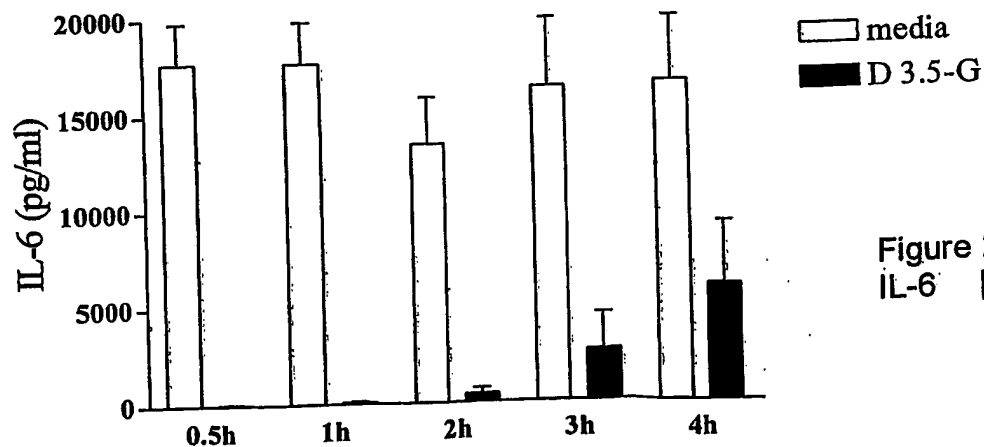


Figure 21(vi)  
IL-6 [n=2]

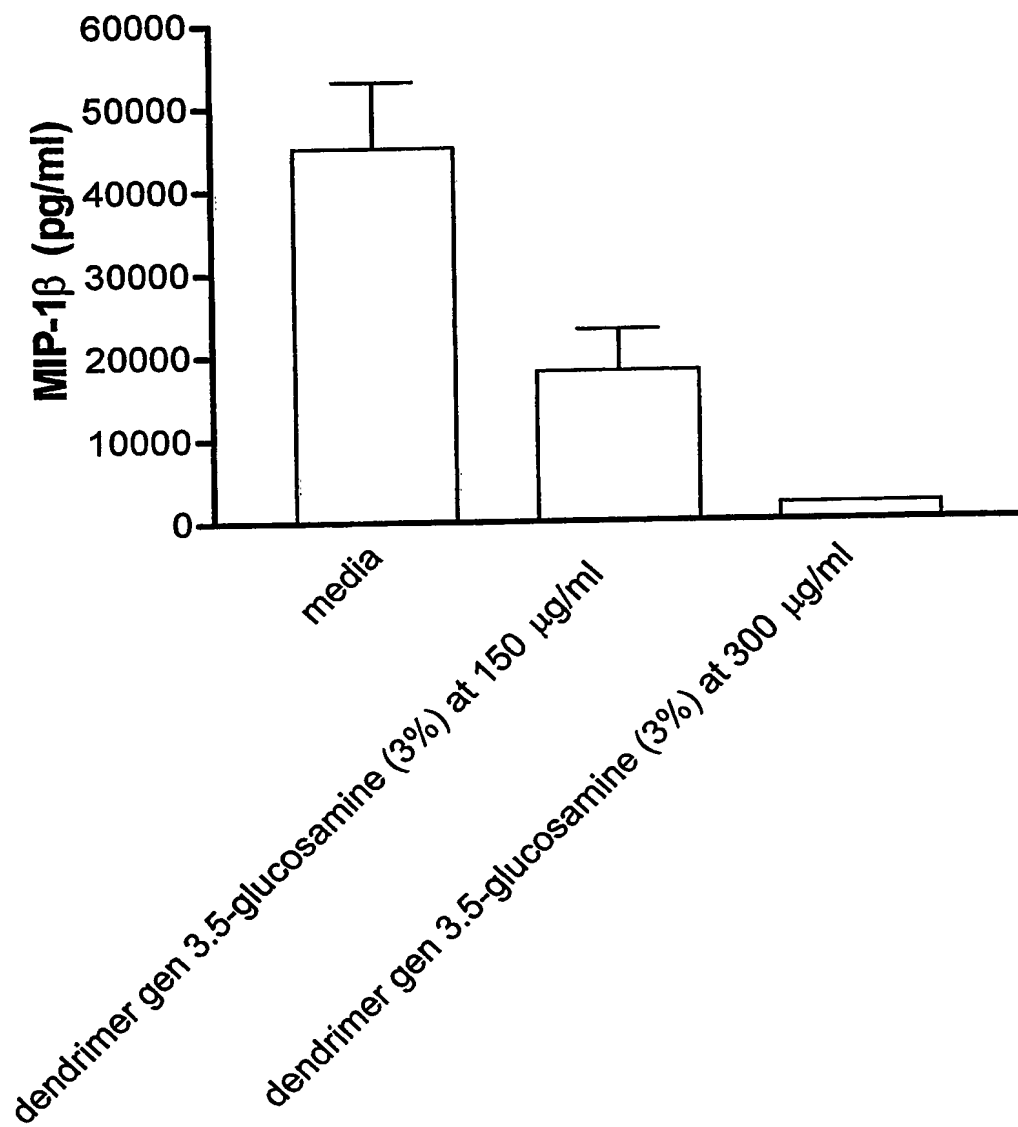


Figure 22(i):

Release of MIP-1 $\beta$  from single donor PBMN cells. LPS (5ng/ml) was added 30 min after the dendrimer generation 3.5-glucosamine (3% loading) at a concentration of either 150  $\mu$ g/ml or 300  $\mu$ g/ml. Culture supernatants were harvested at 22 h (n=1).

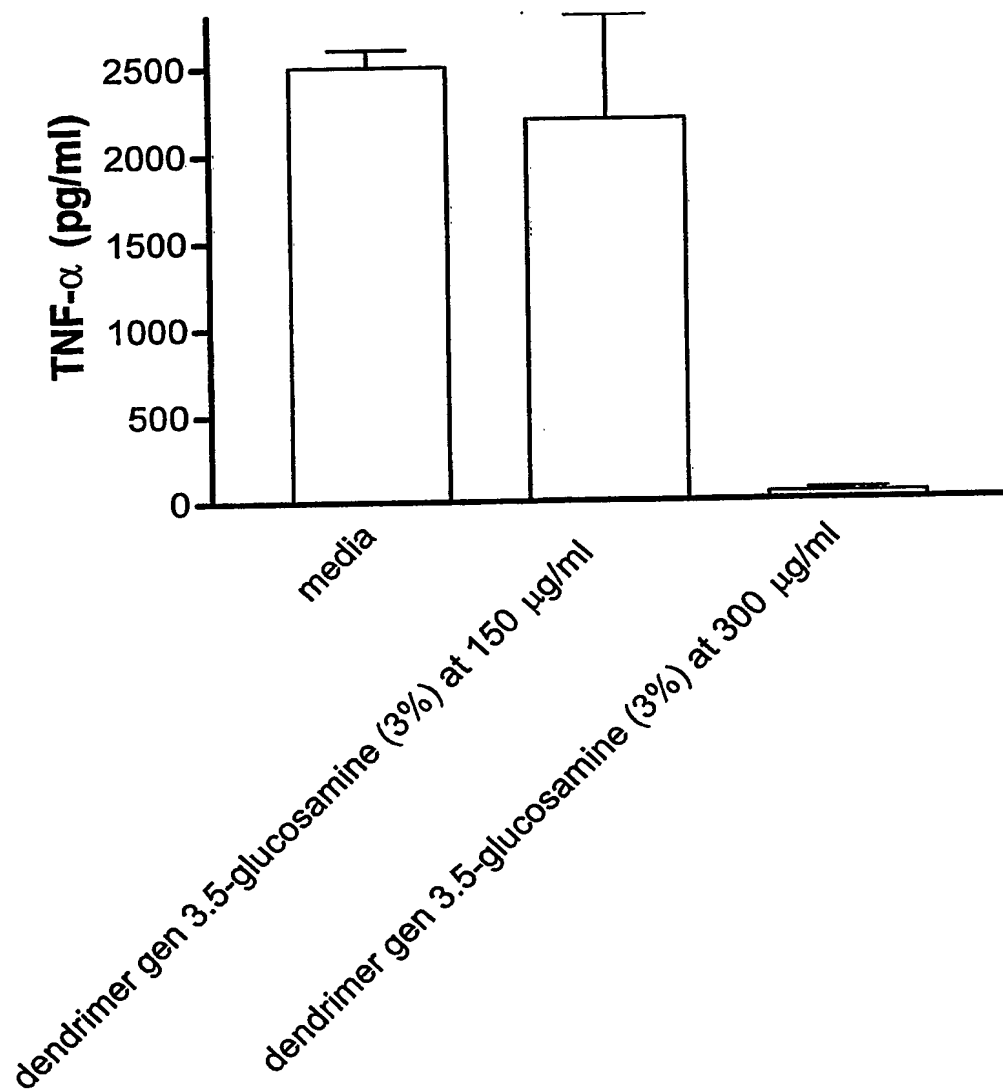


Figure 22(ii):

Release of TNF- $\alpha$  from single donor PBMN cells. LPS (5ng/ml) was added 30 min after the dendrimer generation 3.5-glucosamine (3% loading) at a concentration of either 150  $\mu$ g/ml or 300  $\mu$ g/ml. Culture supernatants were harvested at 22 h (n=1).

Figure 23(i)  
PBMN cells from 3 donors mixed [n=2]

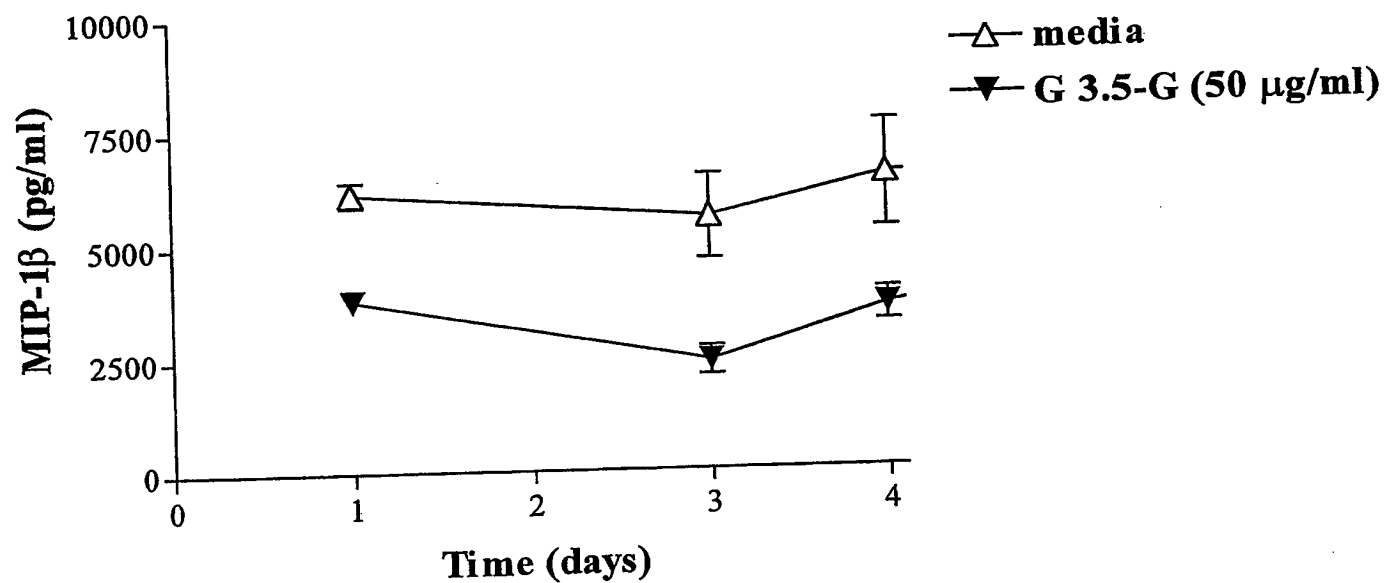




Figure 23(ii)  
PBMN cells from 3 donors mixed [n=2]

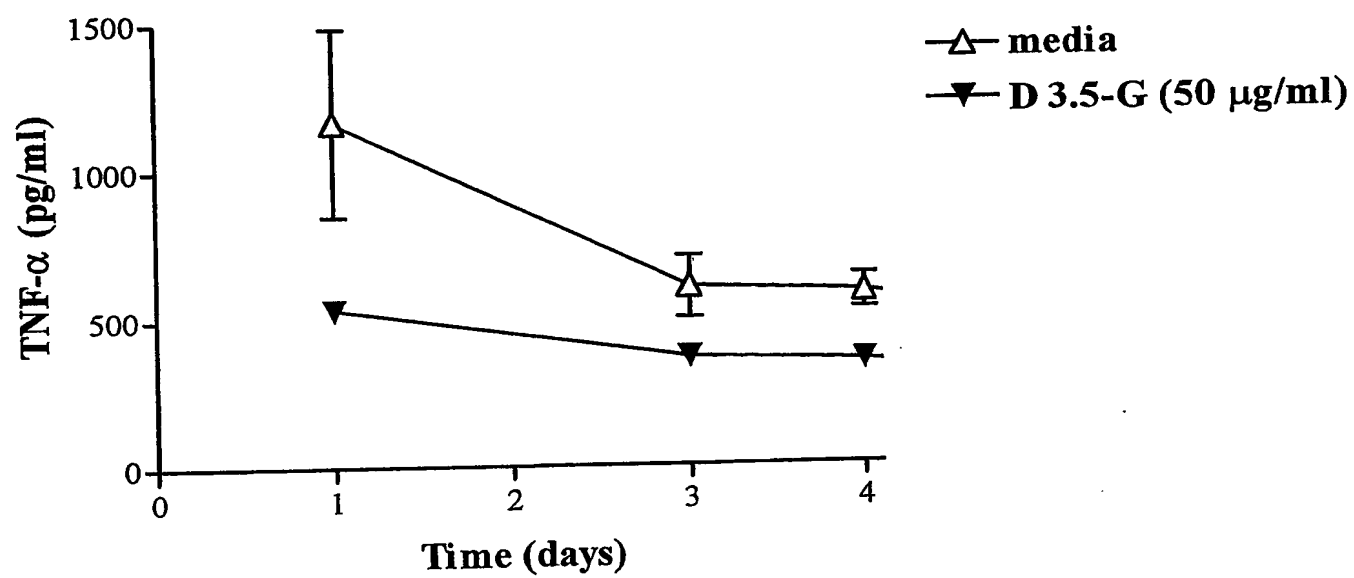


Figure 23(iii)  
PBMN cells from 2 donors mixed [n=2]

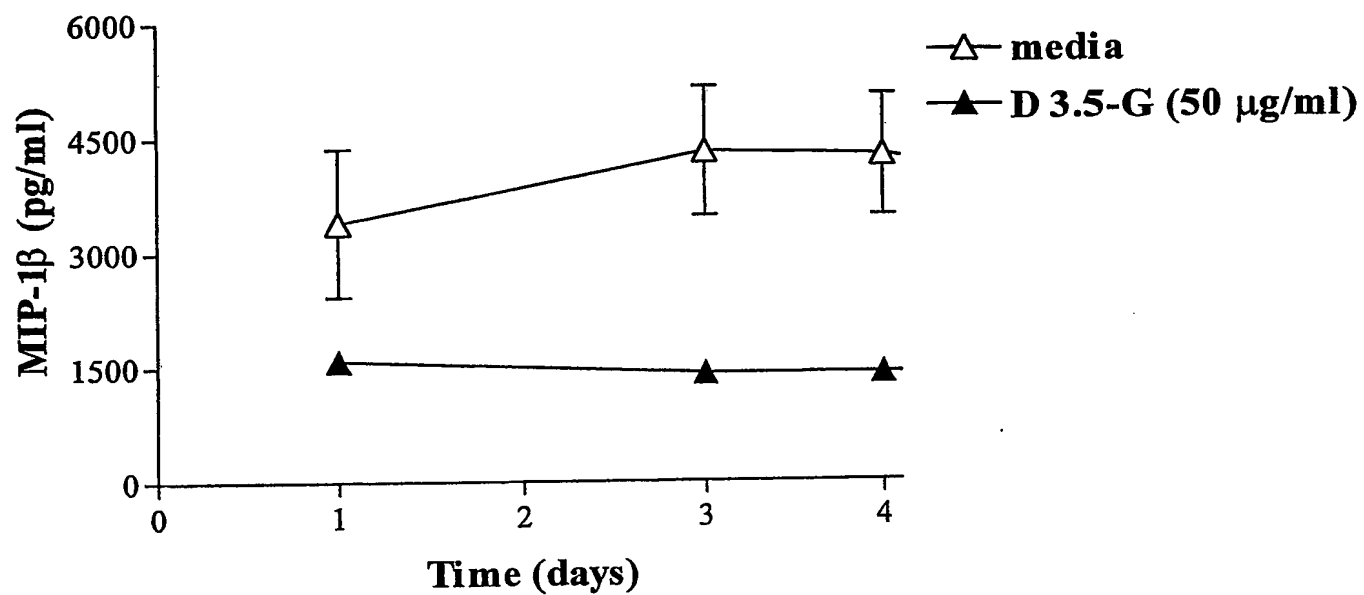
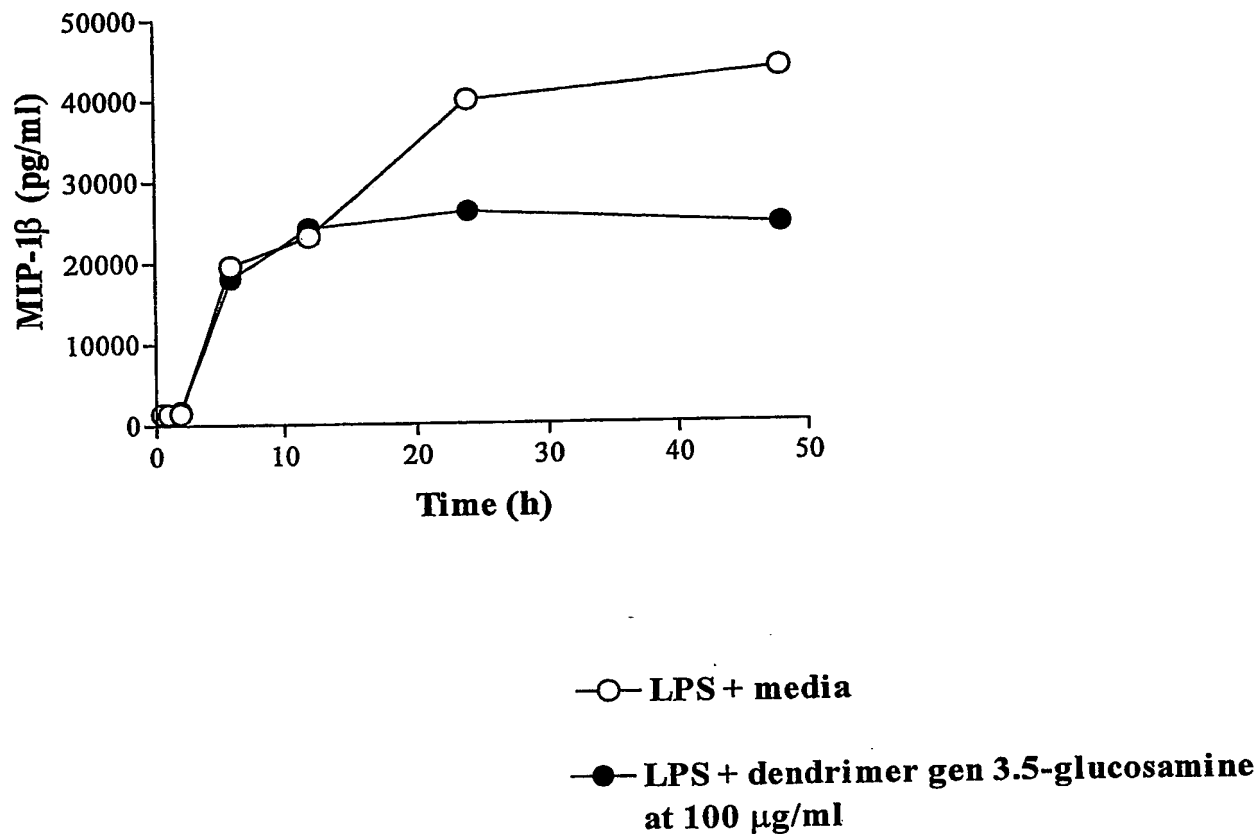
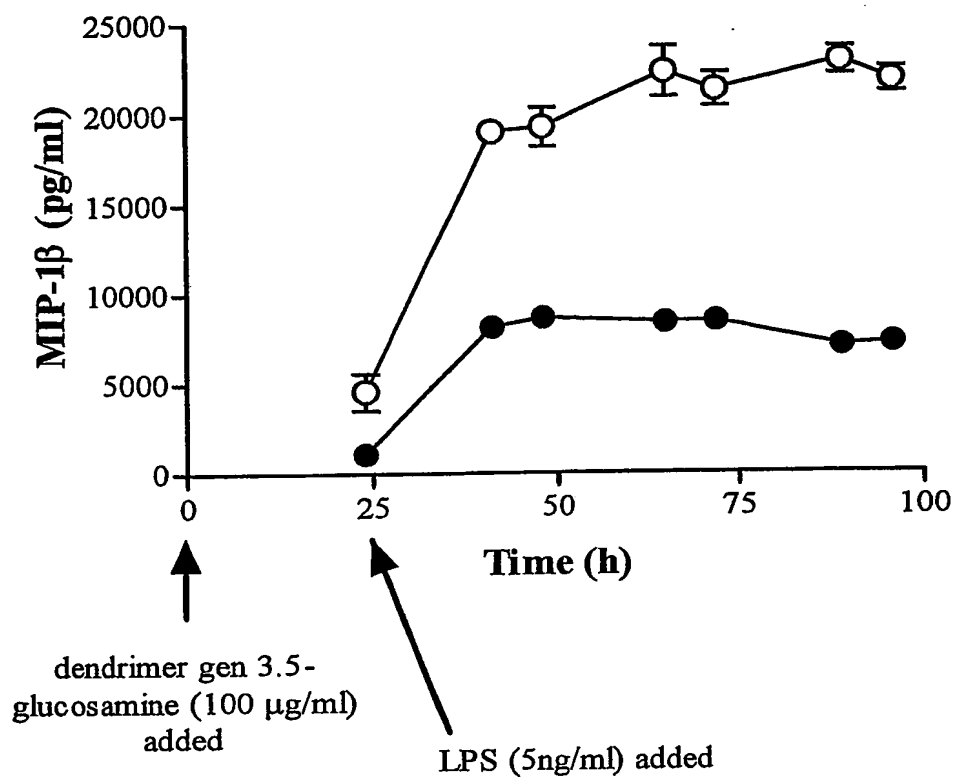


Figure 24(i)  
PBMN cells from 2 donors mixed [n=2]



Donor PBMN cells from 2 individuals mixed,  
Dendrimer gen 3.5-glucosamine (100 μg/ml)  
followed immediately by LPS at 10 ng/ml).

Figure 24(ii)



—○— LPS (5 ng/ml) + media

—●— LPS (5 ng/ml) +  
dendrimer gen 3.5-glucosamine  
at 100 μg/ml

PBMN cells from 2 individuals were mixed and the dendrimer gen 3.5-glucosamine added at 100 μg/ml. 24 hours later, LPS was added at 5 ng/ml (n=2).

Figure 25(i)

PBMN cell viability when cultured with compounds for up to 5 days.

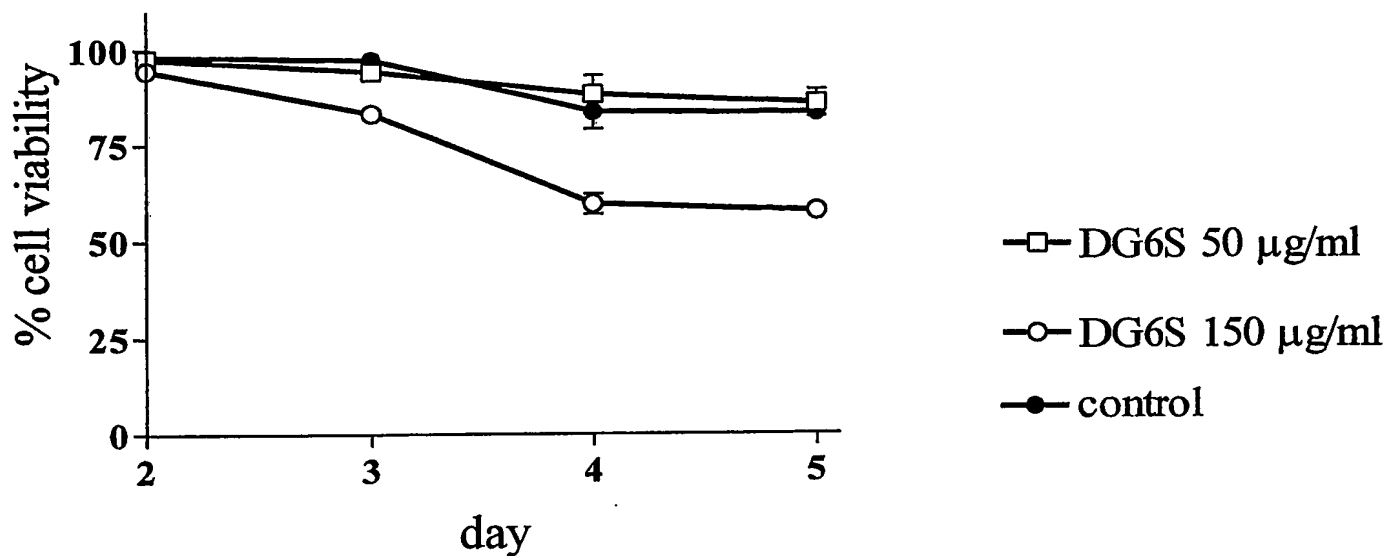


Figure 25(ii)

PBMN cell counts when cultured with compounds for up to 5 days.

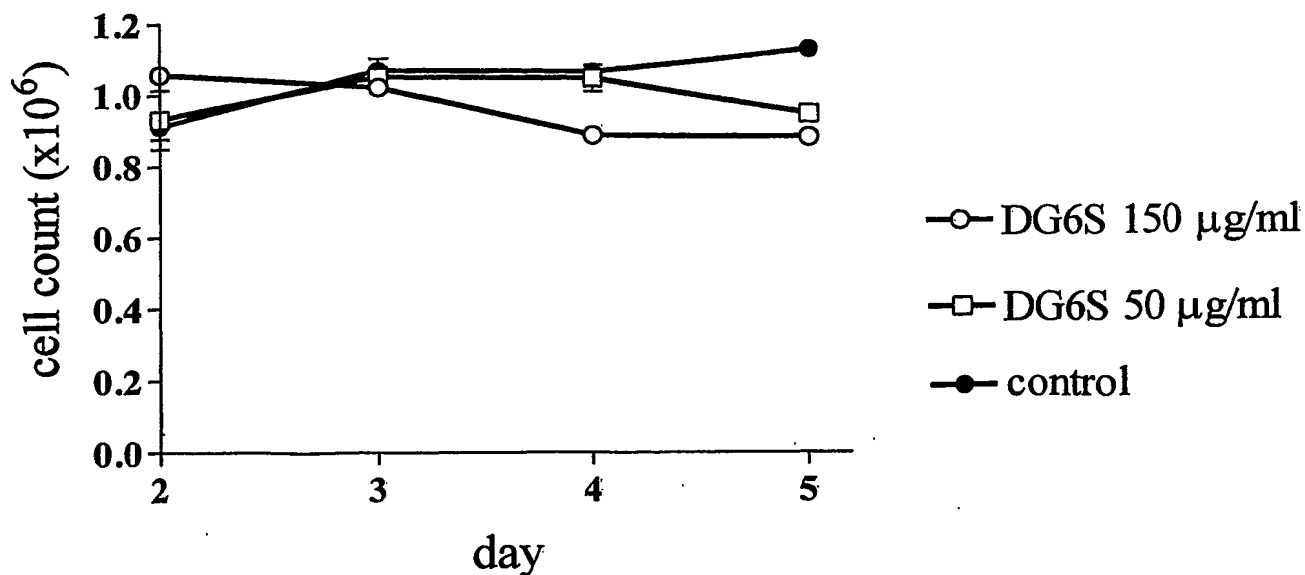
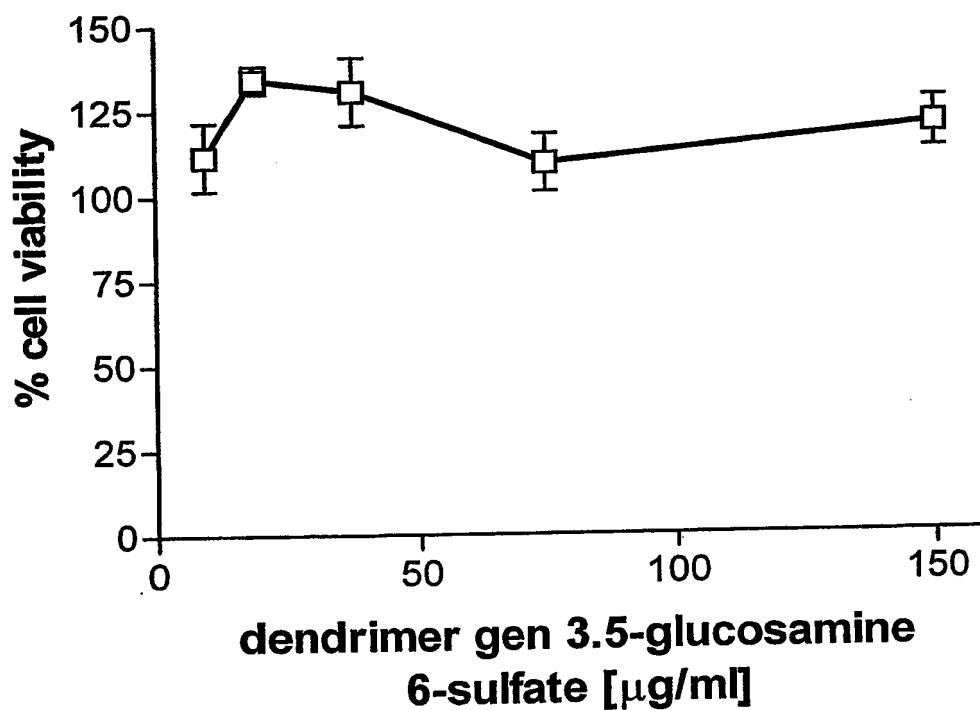


Figure 26:  
Cell viability of MDMs when cultured with  
dendrimer gen 3.5-glucosamine 6-sulfate over a period of 5 days



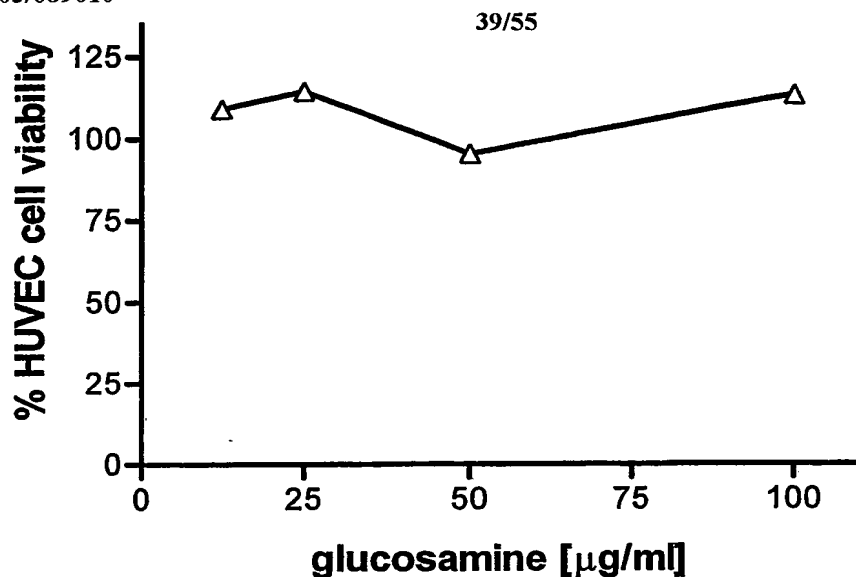


Figure 27 (i)

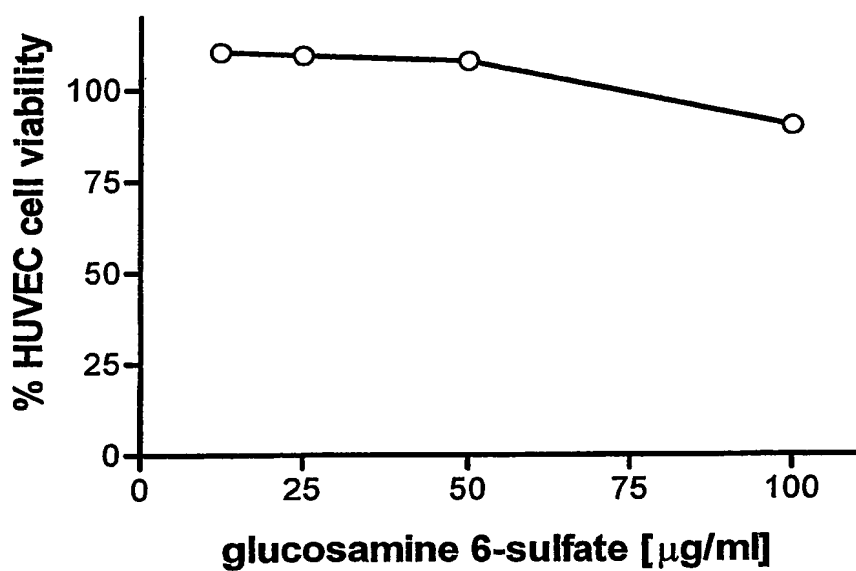


Figure 27 (ii)

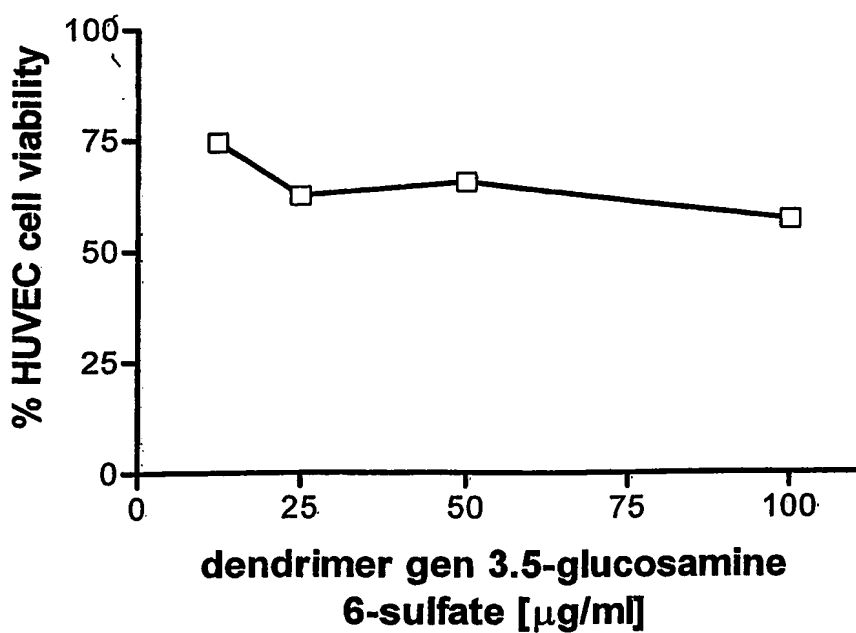


Figure 27 (iii)

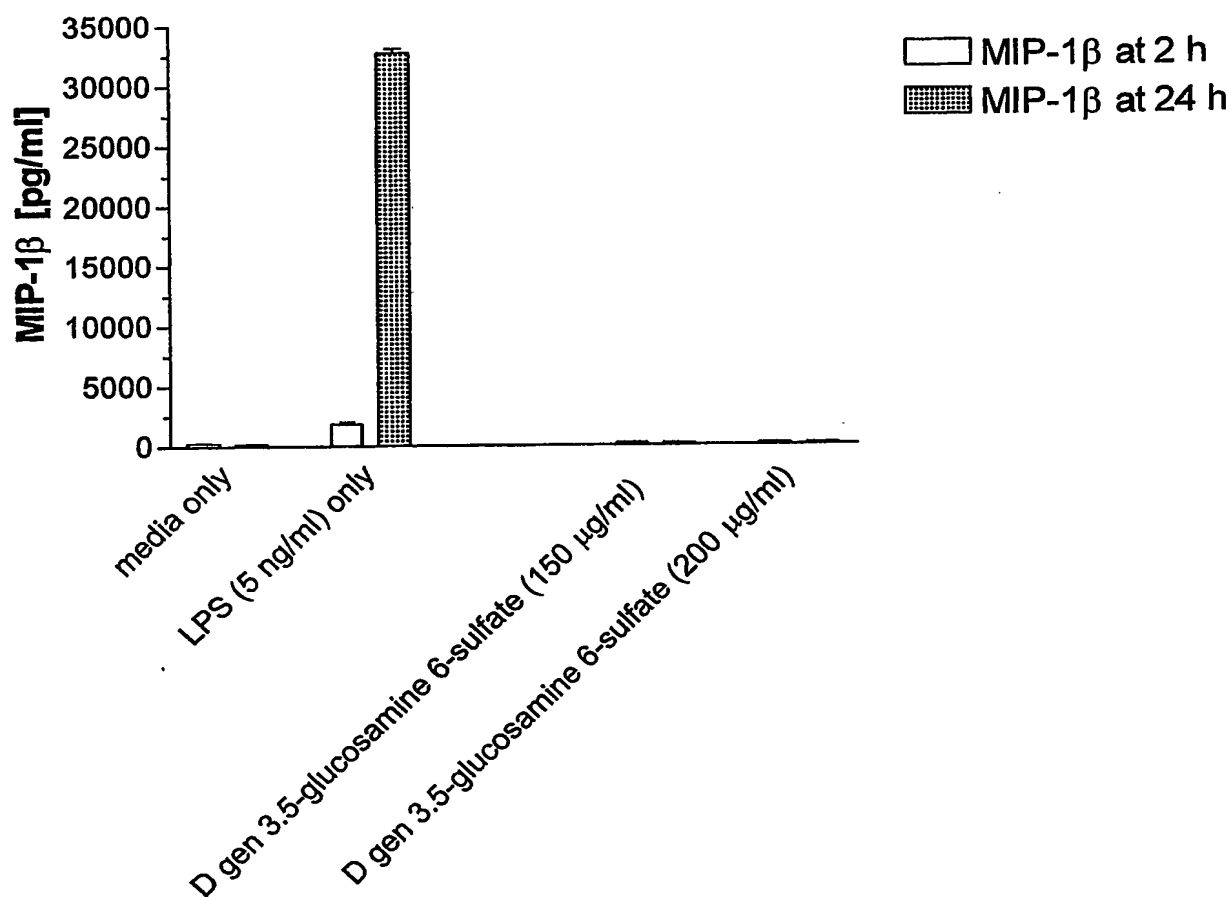


Figure 28(i):  
MIP-1β release from single donor PBMN cells when treated with dendrimer gen 3.5-glucosamine 6-sulfate at 150 μg/ml or 200 μg/ml for 30 min before the addition of LPS at 5 ng/ml.



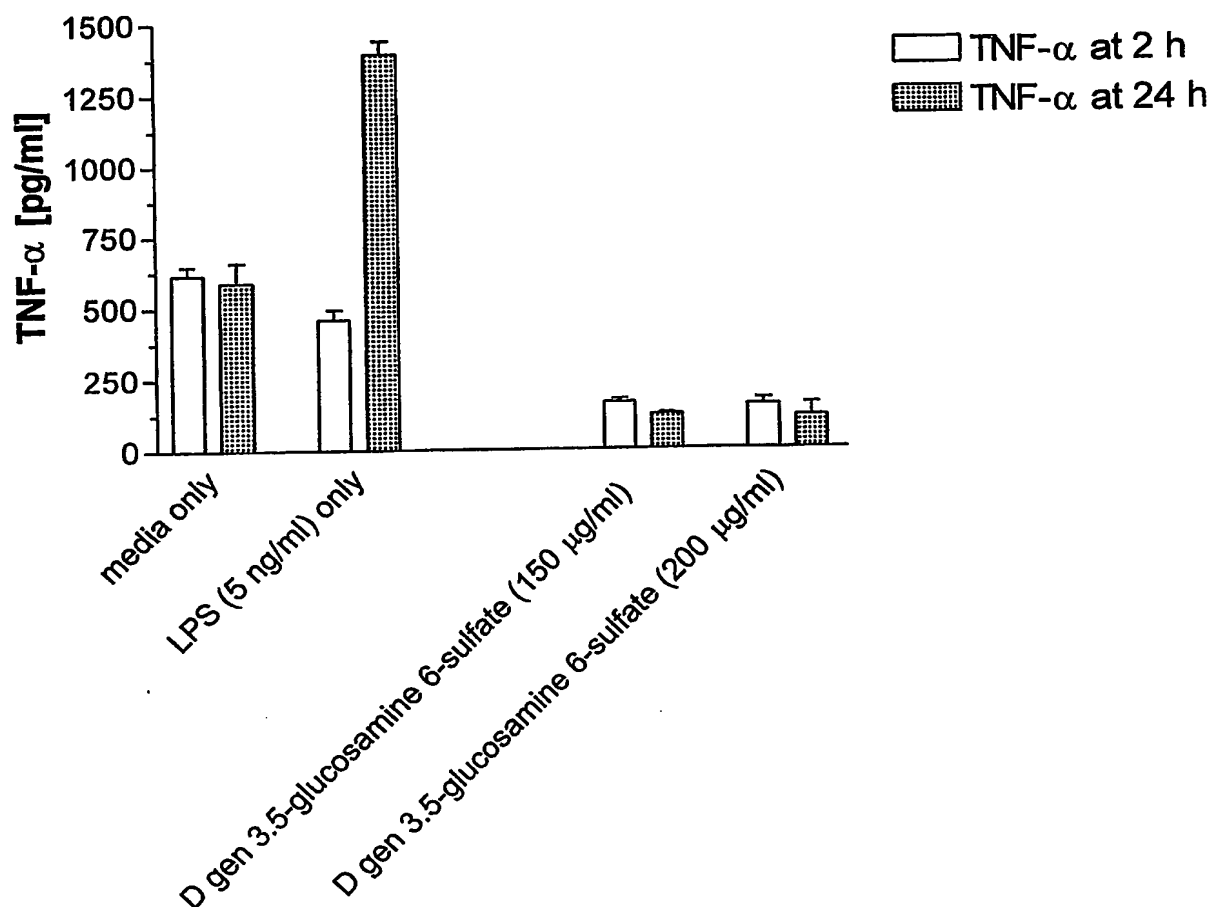


Figure 28(ii):

TNF-α release from single donor PBMN cells when treated with dendrimer gen 3.5-glucosamine 6-sulfate at 150 µg/ml or 200 µg/ml for 30 min before the addition of LPS at 5 ng/ml.

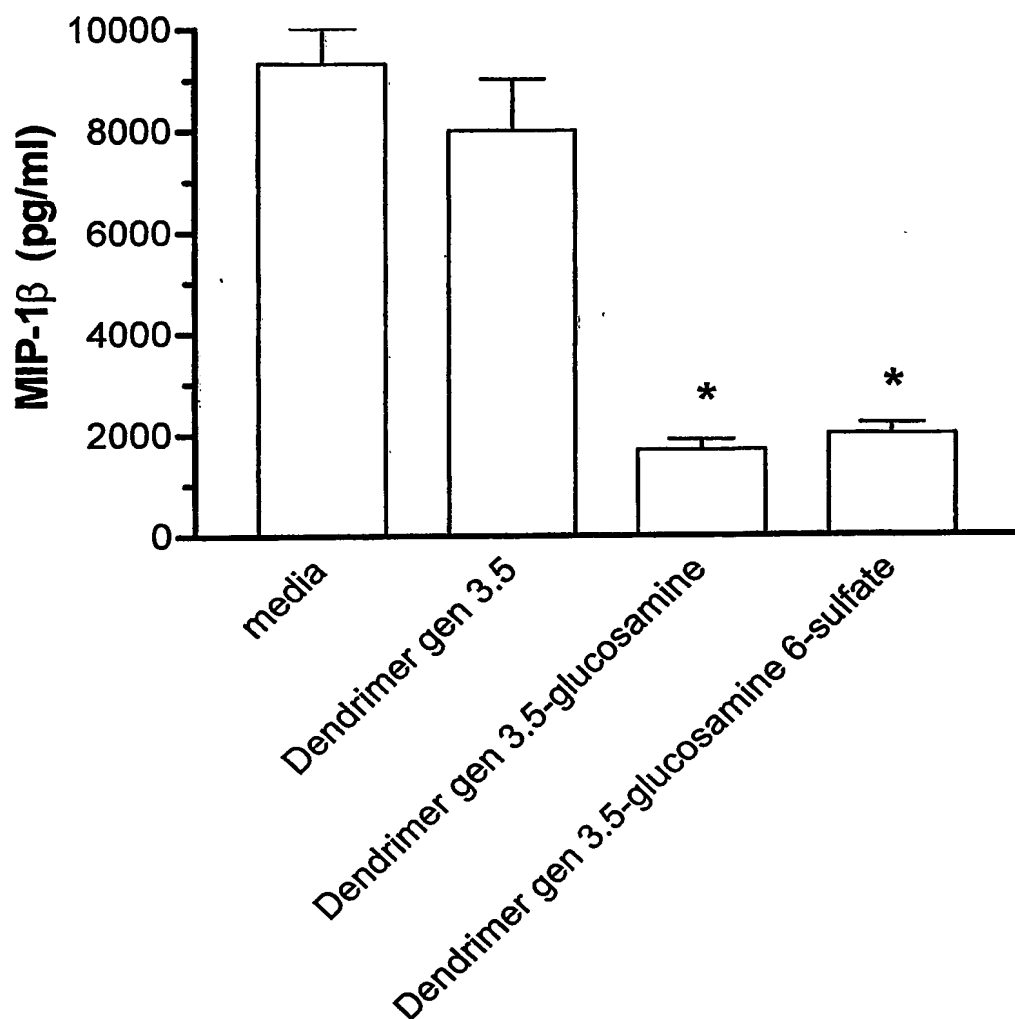


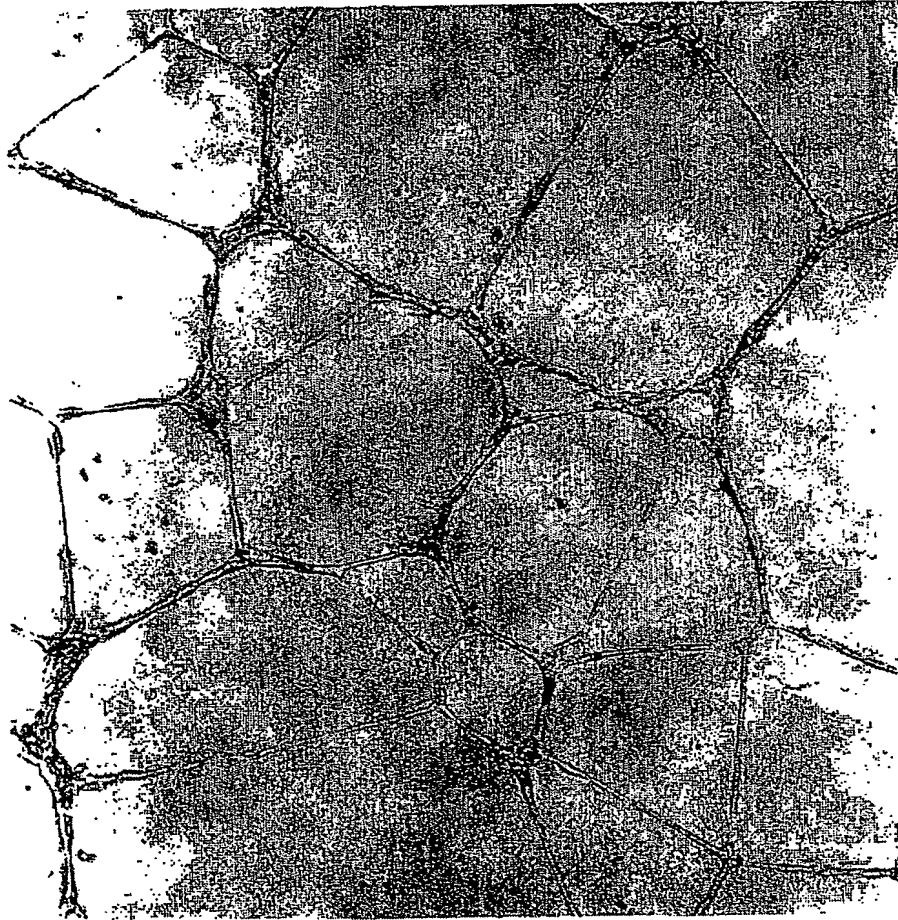
Figure 29:

MDMs from four donors were pooled for 24 h and each of the compounds shown then added at a concentration of 125  $\mu$ g/ml. Cell free culture supernatants were then harvested at 36 h for measurement of MIP-1 $\beta$ . A reduction in MIP-1 $\beta$  was seen when dendrimer gen 3.5 glucosamine or dendrimer gen 3.5-glucosamine 6-sulfate was present.

\*  $p < 0.05$  compared to the media control

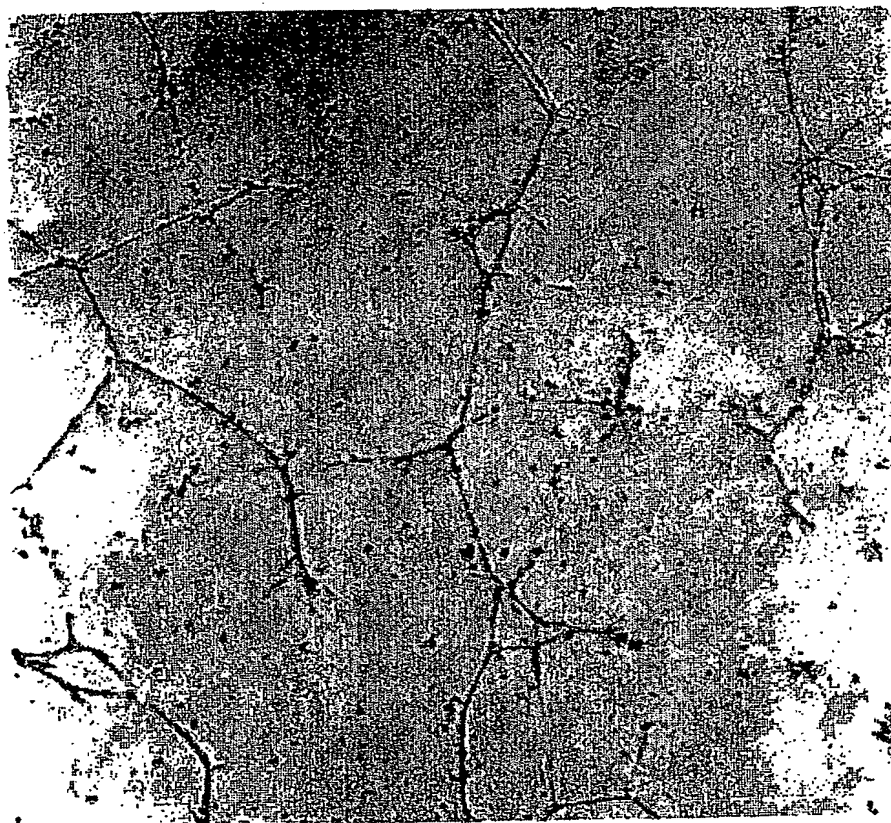
**Figure 30:** Endothelial microtubule formation by HUVECs on Matrigel (x 40 magnification).  
A visual analogue scale was used to determine the extent of tube formation and scored on a scale from 0 (all cells remain single) to 4 (all cells involved in tubular structures) as described in the text.

**Control well**



**Figure 31:** Endothelial microtubule formation by HUVECs on Matrigel (x 40 magnification). A visual analogue scale was used to determine the extent of tube formation and scored on a scale from 0 (all cells remain single) to 4 (all cells involved in tubular structures) as described in the text.

**Dendrimer gen. 3.5-glucosamine 6-sulfate at 12.5 µg/ml**



**Figures 32:** Endothelial microtubule formation by HUVECs on Matrigel (x 40 magnification).  
A visual analogue scale was used to determine the extent of tube formation and scored on a scale from 0 (all cells remain single) to 4 (all cells involved in tubular structures) as described in the text.

**Dendrimer gen 3.5-glucosamine 6-sulfate at 50 µg/ml**

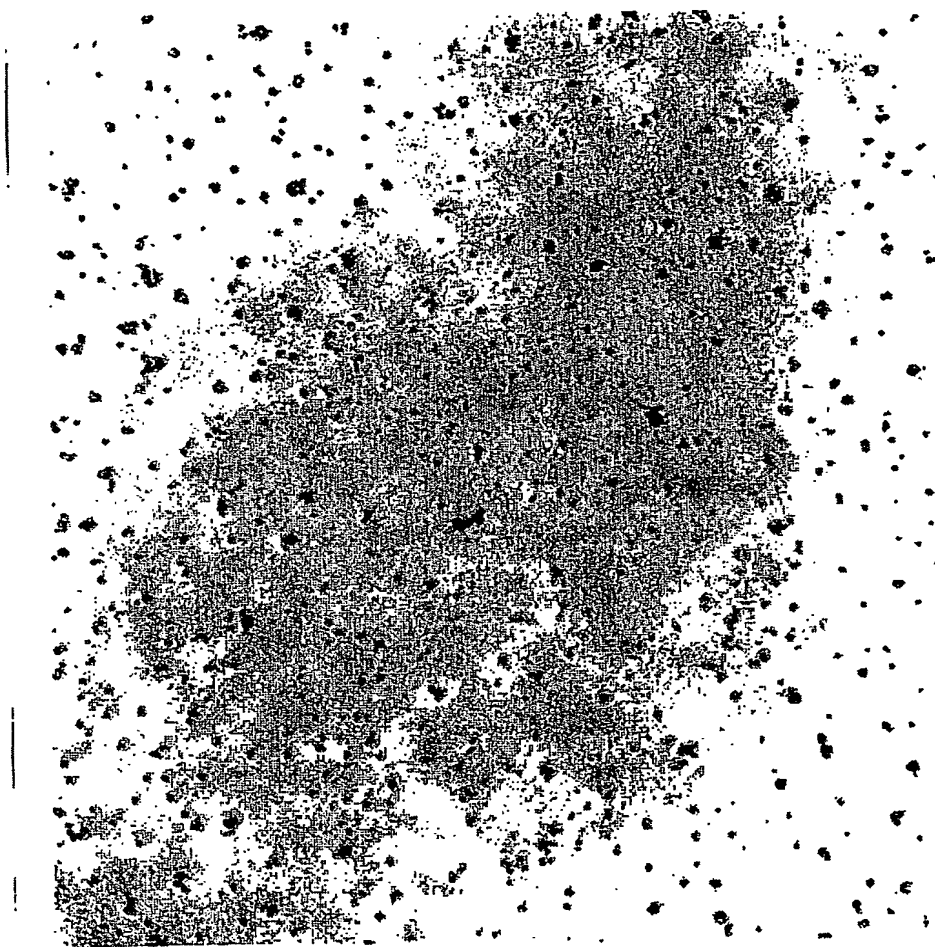
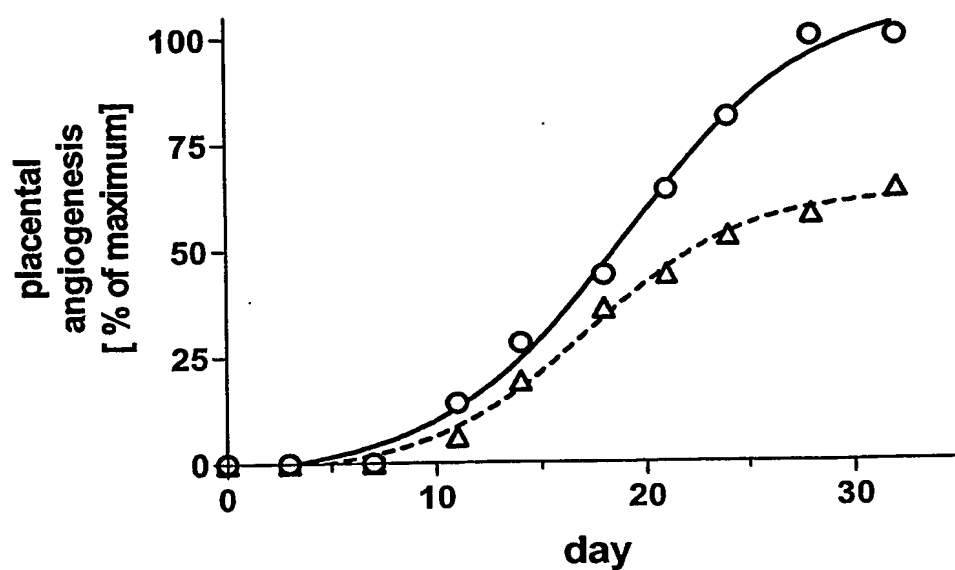


Figure 33: Dendrimer gen. 3.5 glucosamine 6-sulfate reduced the rate of new blood vessel formation in an *in vitro* human placental angiogenesis assay.



○ Control

△ Dendrimer gen. 3.5 glucosamine 6-sulfate (50 µg/ml)

# New treatments for inflammatory diseases

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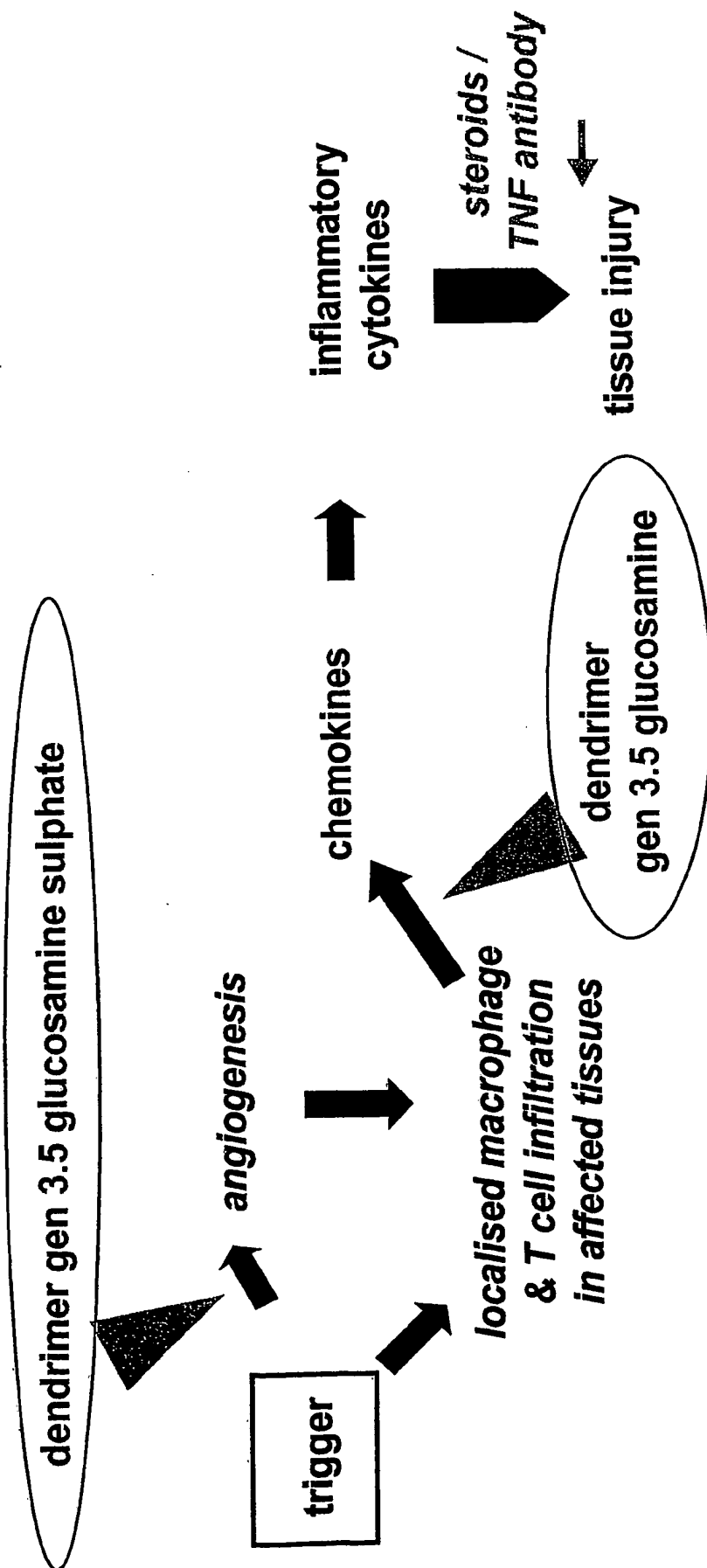


Figure 34

Figure 35:

Release of MIP-1 $\beta$  and TNF- $\alpha$  from single donor monocyte derived dendritic cells that were exposed to LPS (5 ng/ml) in the absence and the presence of dendrimer gen. 3.5 glucosamine (D 3.5-G) at 200  $\mu$ g/ml.

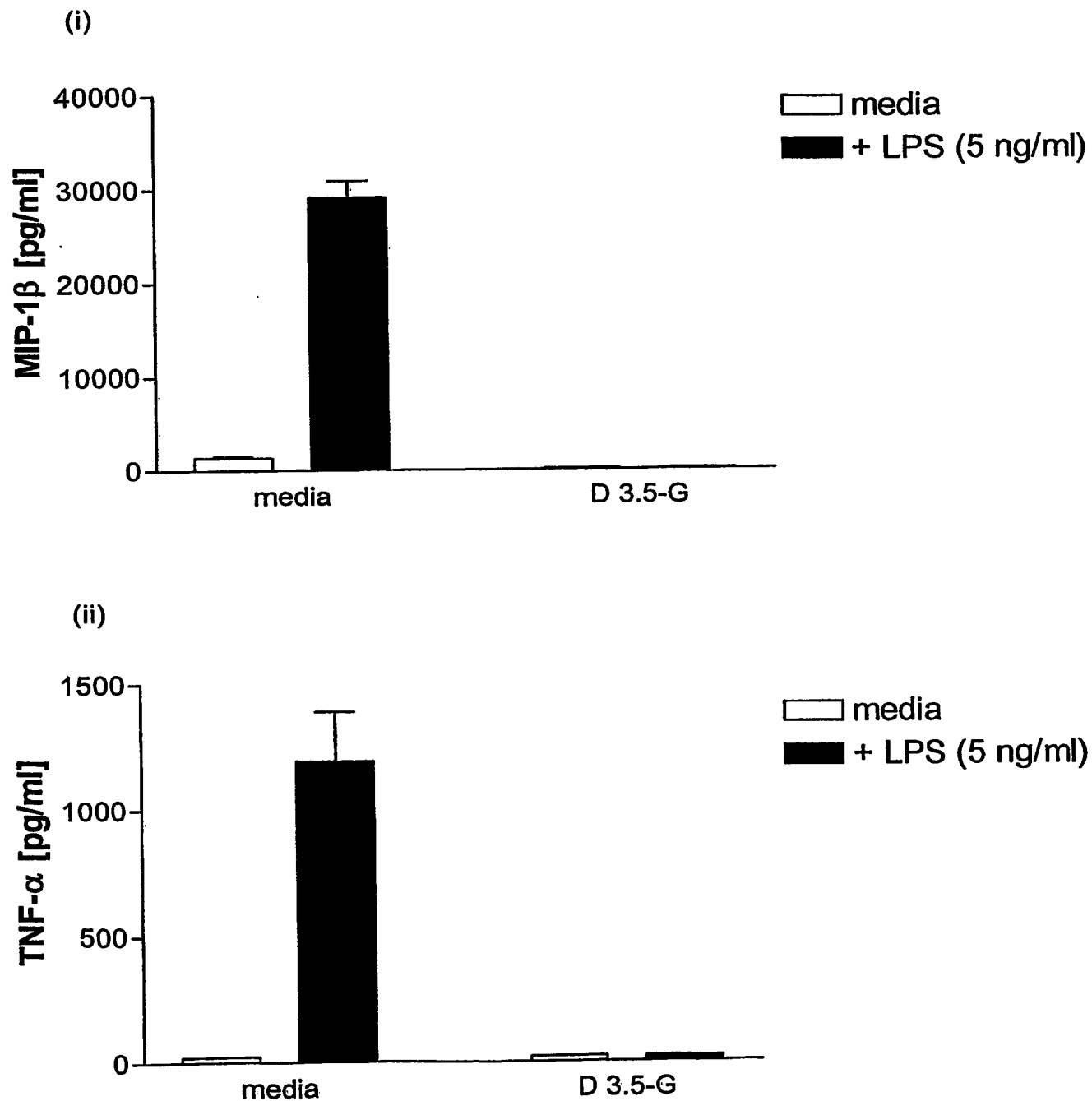
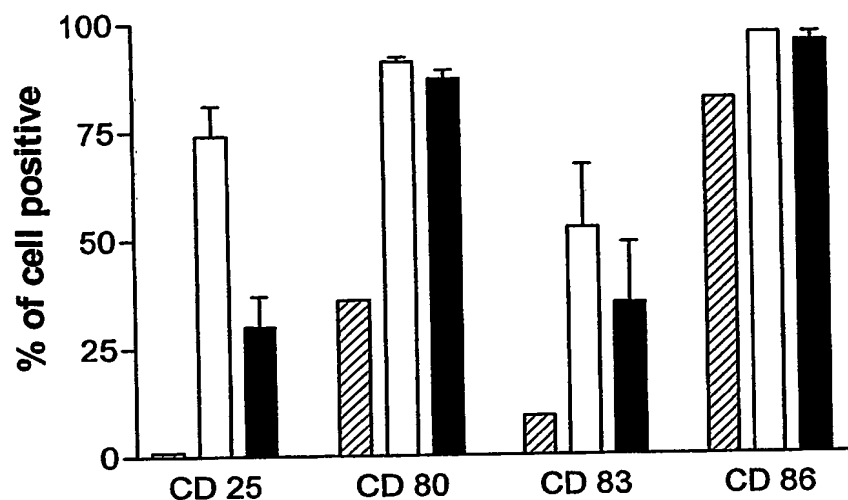




Figure 36:

Effect of dendrimer gen. 3.5-glucosamine (200  $\mu$ g/ml) on the upregulation of CD 25, CD 80, CD 83 and CD 86 on monocyte derived dendritic cells by LPS (5 ng/ml).



Media control

LPS (5 ng/ml)

Dendrimer gen. 3.5-glucosamine (200  $\mu$ g/ml) + LPS (5 ng/ml)

Figure 37: Effect of dendrimer gen. 3.5 glucosamine (D 3.5-G at 200  $\mu\text{g/ml}$ ) on the allogeneic mixed lymphocyte reaction using monocyte derived dendritic cells (MDDCs) in the absence & the presence of LPS (5 ng/ml).

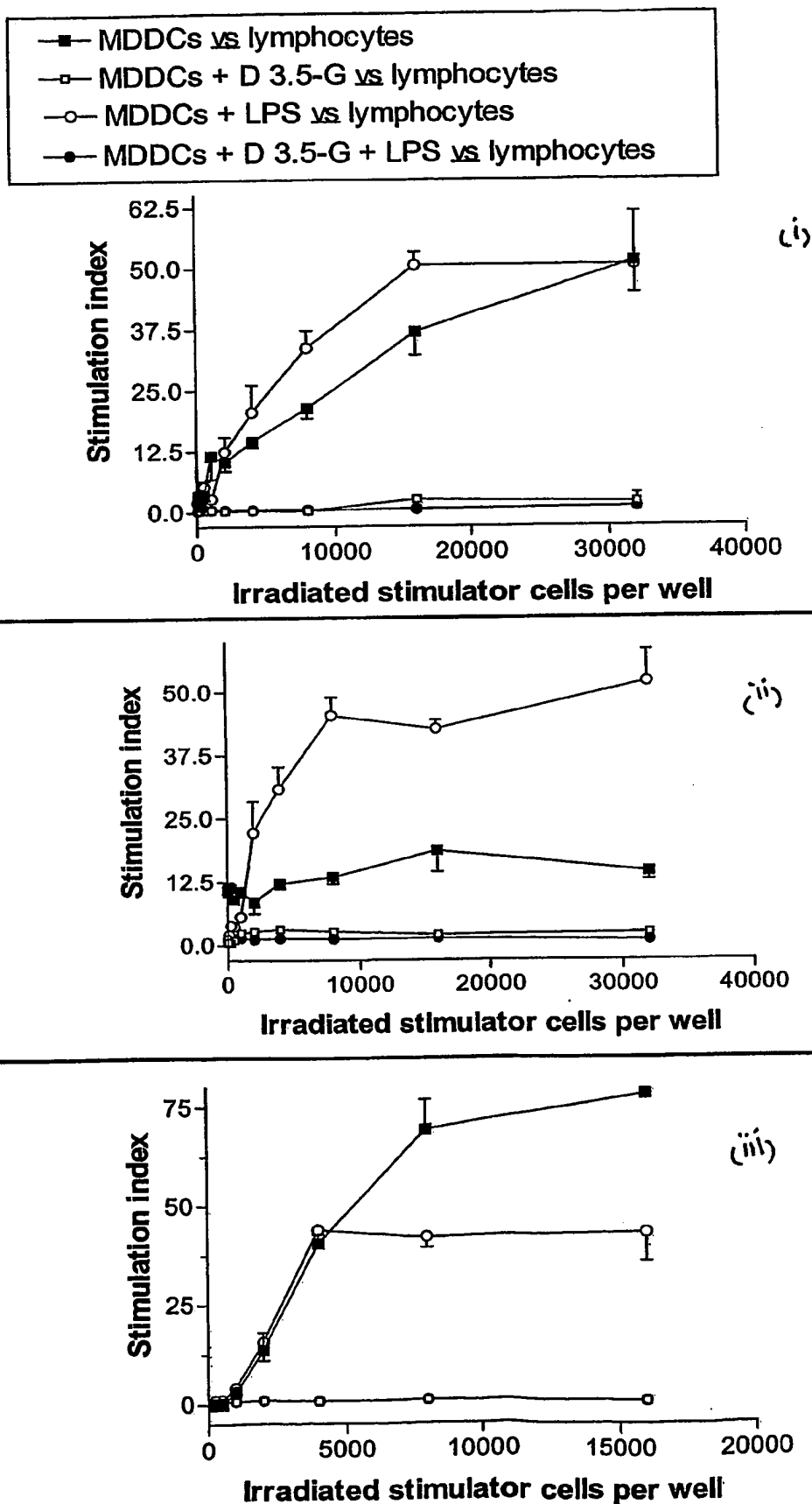


Figure 38: Effect of dendrimer gen. 3.5 glucosamine (D 3.5-G at 200  $\mu\text{g/ml}$ ) on the allogeneic mixed lymphocyte reaction using monocyte derived dendritic cells (MDDCs) in the absence and the presence of LPS (20  $\text{ng/ml}$ ).

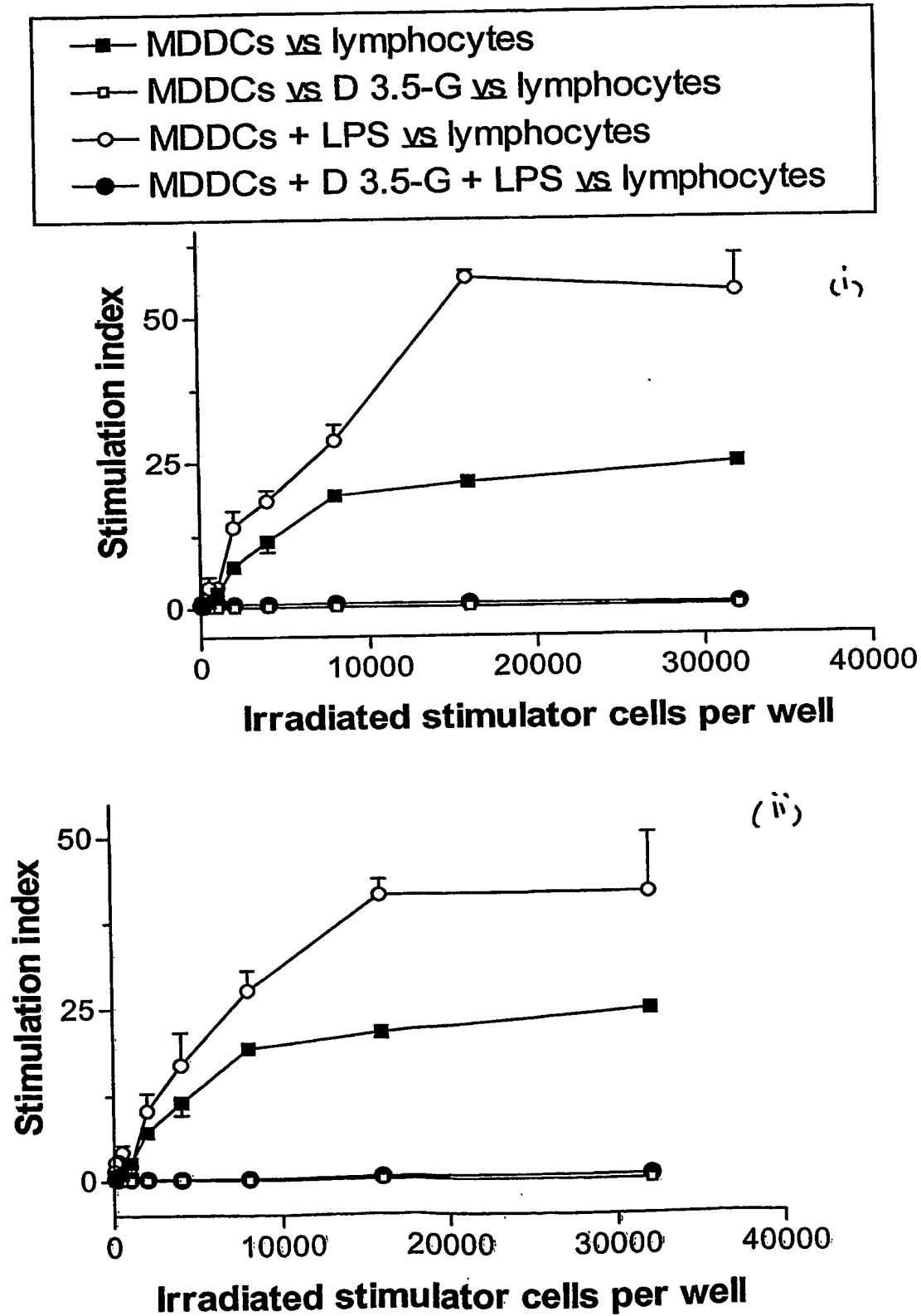


Figure 39: Effect of dendrimer gen. 3.5 glucosamine (D 3.5-G at 200  $\mu\text{g/ml}$ ) on the allogeneic mixed lymphocyte reaction using peritoneal dendritic cells in the absence & presence of LPS (5  $\text{ng/ml}$ ).

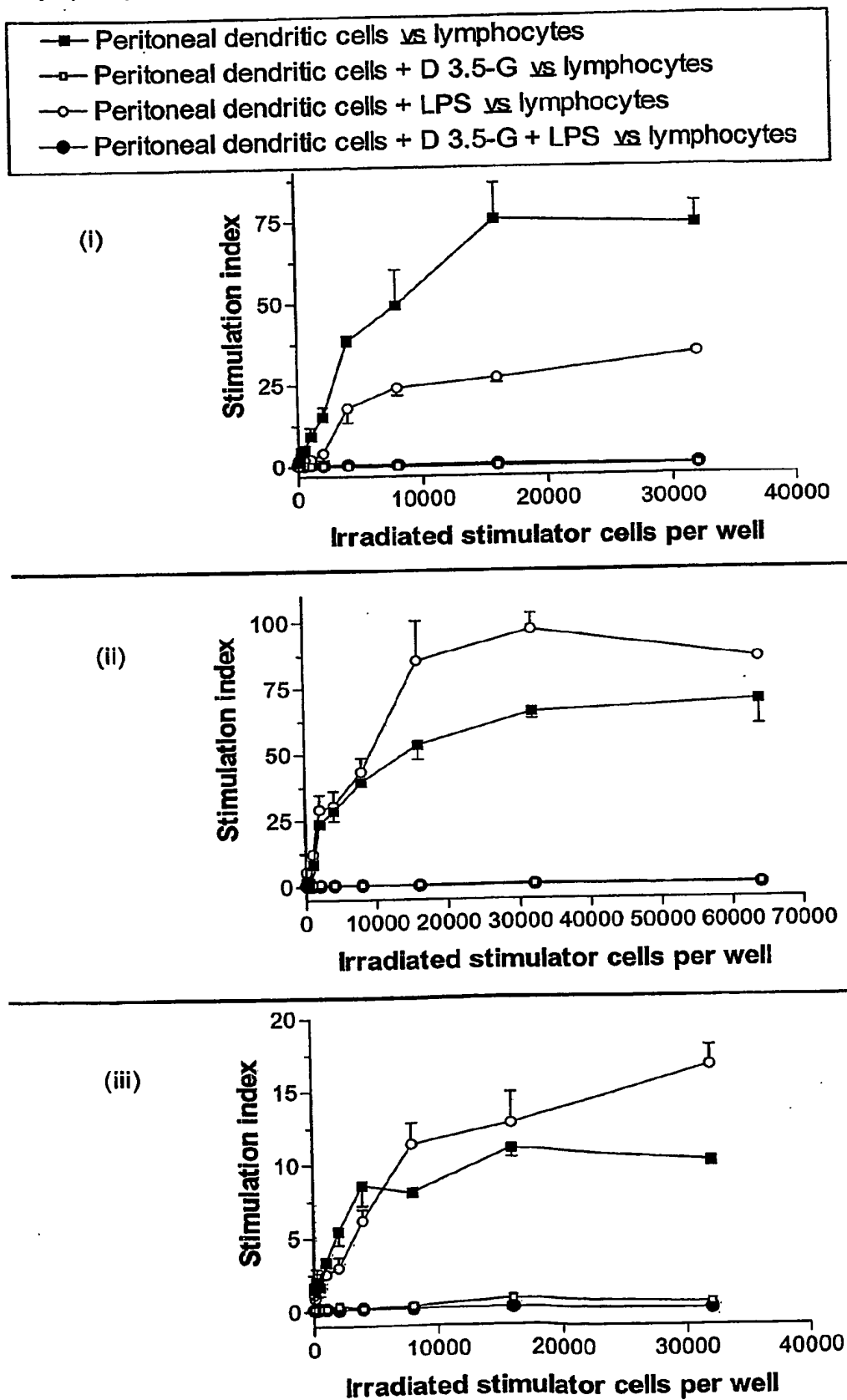
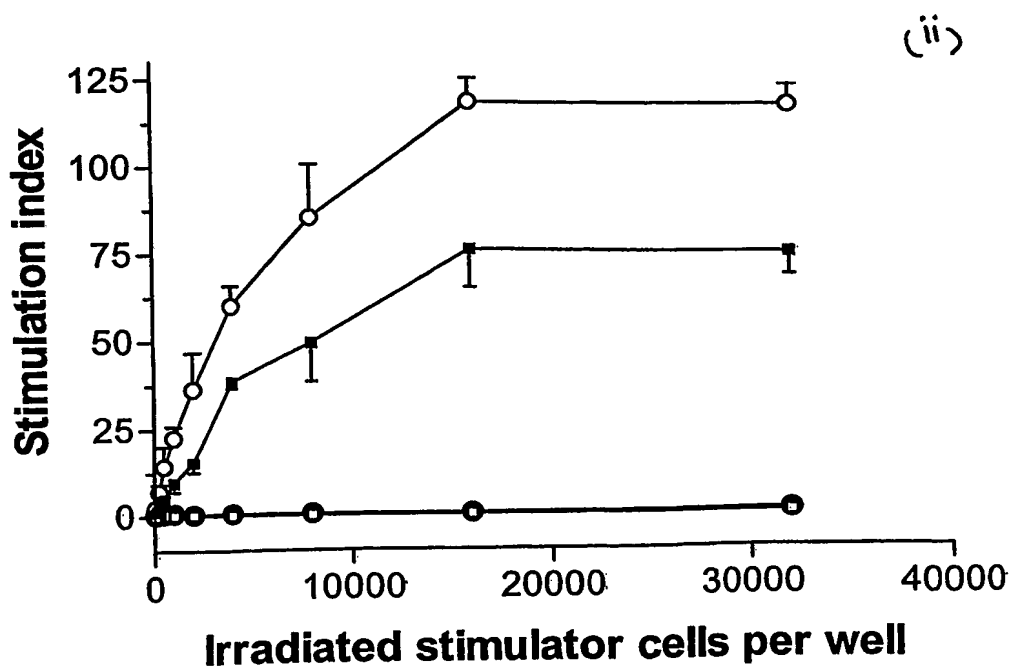
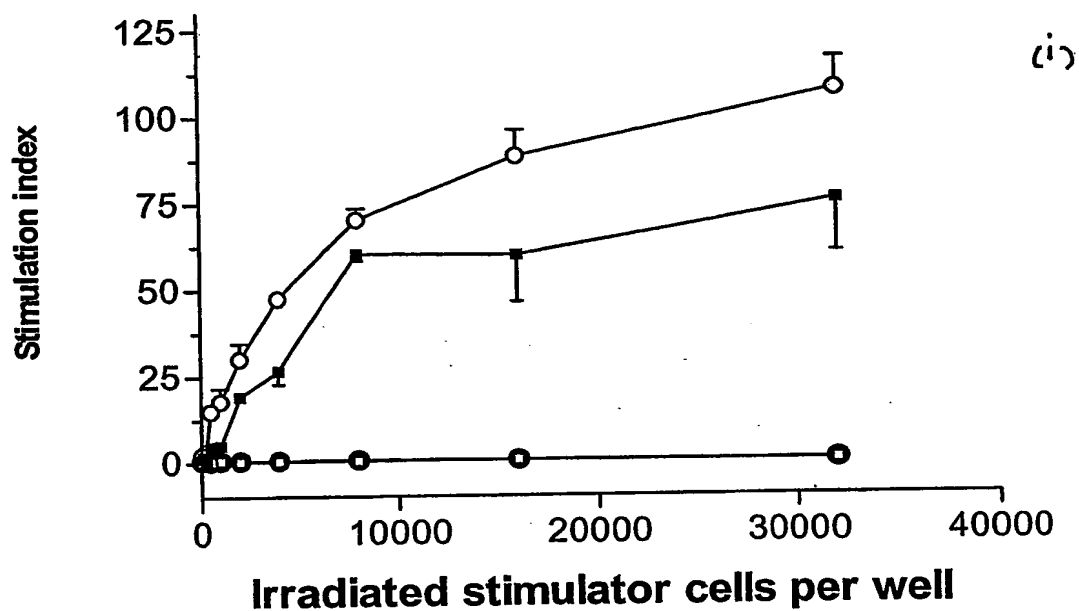
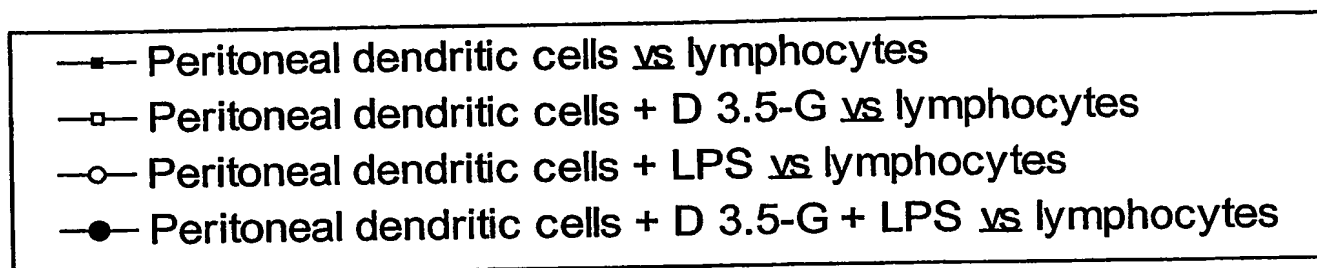
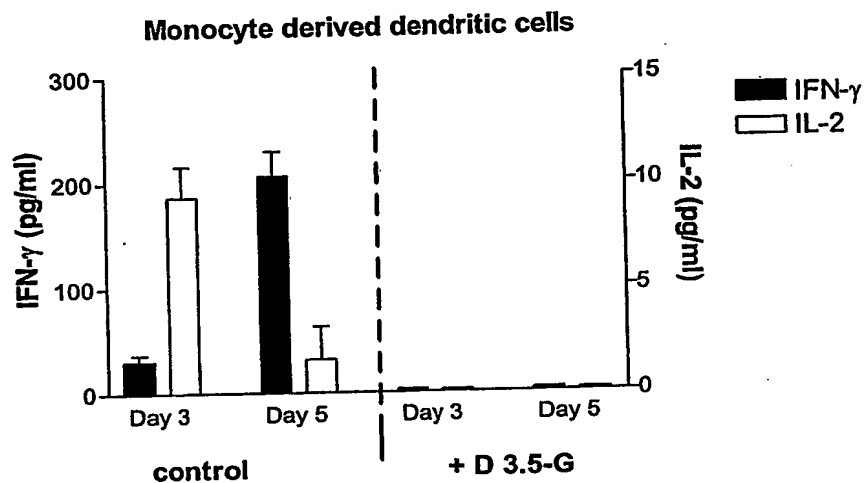
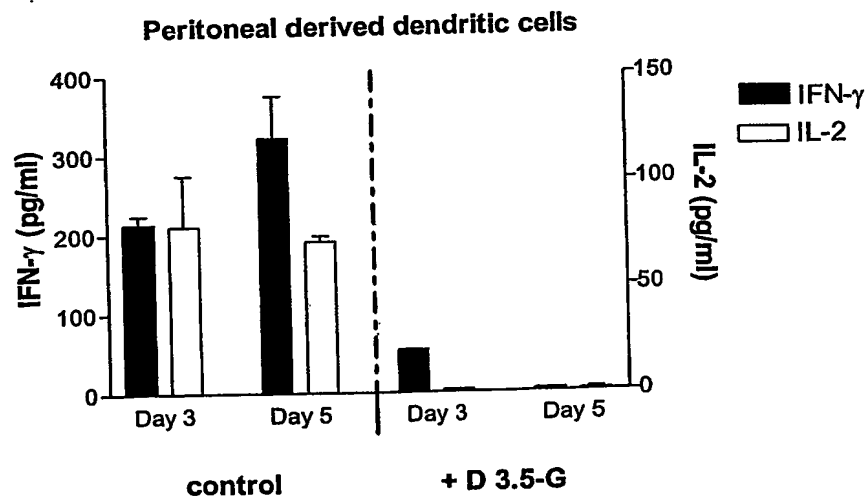


Figure 40: Effect of dendrimer gen. 3.5 glucosamine (D 3.5-G at 200  $\mu\text{g/ml}$ ) on the allogeneic mixed lymphocyte reaction using peritoneal dendritic cells in the absence and the presence of LPS (20 ng/ml).



**Figure 41:****(i)**

32,000 monocyte derived dendritic cells were stimulated with LPS (20 ng/ml) for 21 h, washed and incubated with 100,000 allogeneic lymphocytes. Cell free supernatants were then analysed at days 3 and 5. (D 3.5-G = dendrimer gen. 3.5 glucosamine)

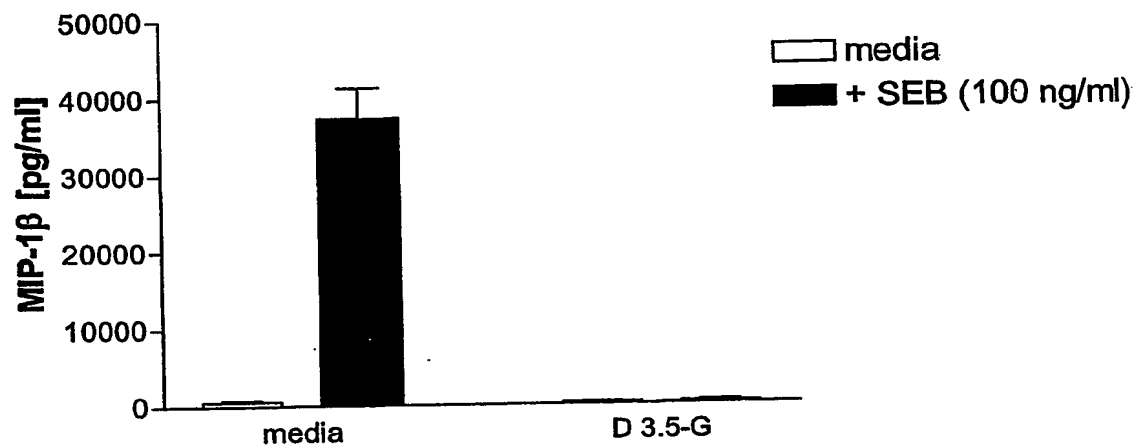
**(ii)**

32,000 peritoneal dendritic cells were stimulated with LPS (20 ng/ml) for 21 h, washed and incubated with 100,000 allogeneic lymphocytes. Cell free culture supernatants were then analysed at days 3 and 5.

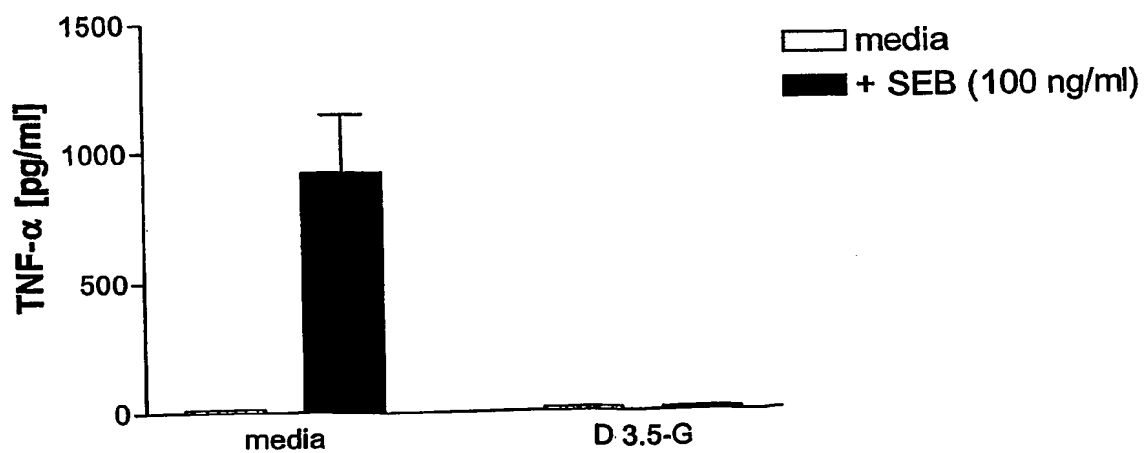
Figure 42:

Release of MIP-1 $\beta$  and TNF- $\alpha$  from single donor PBMN cells that were exposed to SEB (100 ng/ml) in the absence and the presence of dendrimer gen 3.5 glucosamine (D 3.5-G) at 200  $\mu$ g/ml.

(i)



(ii)



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